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THE DELTA CELL OF THE HUMAN ADENOHYPOPHYSIS: ITS RESPONSE TO ACUTE AND CHRONIC ILLNESS*

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ABSTRACT

The delta cell of the human adenohypophysis is a sub-type of basophil that can be distinguished easily with the iron-PAS stain. It stains blue-purple in contrast to the red beta cell.

In a necropsy study of pituitary cytology in the human subject, it was found that the percentage of delta cells changed significantly with increasing duration of the terminal illness, while the other cell types remained relatively constant. In patients dying within twenty-four hours of the onset of the final illness, the average percentage of delta cells was 8.5. Illnesses lasting longer than two weeks were accompanied by a decrease in the percentage of delta cells to 1.9. In an intermediate group of patients whose terminal disease lasted from twenty-four hours to fourteen days, the average percentage of delta cells was 4.5.

This temporal pattern of degranulation in acute illness, and the absence of delta cells in the pituitaries of children and pregnant women, suggest that the human delta cell is the source of one or more of the pituitary gonadotropins.

INTRODUCTION

THE periodic-acid-Schiff (PAS) stain was introduced (1, 2) as a histochemical method for staining carbohydrates in fixed tissues. The application of this technique to the anterior pituitary gland has shown that

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only the basophils have large amounts of PAS-positive granulation (3). However, many lightly granulated cells that with standard techniques are classified as chromophobes also take up the PAS stain, but to a lesser degree. Pearse attributed the secretion of carbohydrate-containing hormones to this combined group of cells, which he named the "mucoid" series (4–6).

The anterior pituitary hormones are protein in nature. The existence of 6 principles is generally accepted (7), but there is still some doubt as to whether these substances are manufactured and secreted separately (8). Growth hormone and prolactin are simple proteins and adrenocorticotropic hormone (ACTH) is a polypeptide. Follicle-stimulating hormone, luteinizing hormone and thyrotropic hormone are known to contain appreciable amounts of carbohydrate, and this has been used as evidence that they are manufactured by PAS-positive cells (9, 10). However, the secretion of ACTH, which has no carbohydrate groupings, has also been attributed to the basophils (11–13). It has been suggested that ACTH may be linked in the cell with a carbohydrate-containing complex (9).

As 4 of these anterior pituitary hormones have been associated with PAS-positive cells, it seemed reasonable to enquire whether more than one type of PAS-positive cell might be present in the human pituitary gland. By combining methyl blue with the PAS technique, we were able to demonstrate 2 types of PAS-positive cells, one staining red and the other blue-purple (14). Since then we have found that an iron-PAS procedure, originally described by Ritter and Oleson (15), is more easily performed and yields a better color contrast. With this newer method we have been able to draw a further distinction between a heavily granulated and a lightly granulated blue-purple cell; these we have named the "delta" cell and the "gamma" cell, respectively. The name "beta" has been retained for the cell with red PAS-positive granules, and "alpha" for the cell with PAS-negative granules which takes up the orange G counterstain. Cells without obvious granulation are referred to as chromophobes.

This study is mainly concerned with the "delta" cell. Part A deals with the staining reactions of the delta cell which helped to establish it as a cell with distinctive staining properties. Part B deals with the behavior of this cell in relation to other cell types in normal and pathologic states.

As the stress of illness frequently changes hormonal activity, it was suspected that the histologic pattern of the anterior pituitary might be altered by the nature of the terminal illness, and that strictly "normal" glands would be found only in patients who died suddenly. A preliminary survey of pituitaries obtained at autopsy from routine cases indicated that the greatest numbers of delta cells were found in patients who died suddenly, and that these cells were usually reduced in number in patients who died after lengthy illnesses. A statistical comparison of differential cell counts

was made in the following 3 groups: 1) sudden death, 2) short acute illness, and 3) prolonged debilitating disease. The results confirmed the original observations.

Part A. Staining reactions of the delta cell and the other anterior pituitary cells

MATERIAL AND METHODS

Materials

Pituitary glands obtained from 1,500 unselected routine necropsy cases at the Toronto General Hospital and the Hospital for Sick Children, Toronto, were stained by the iron-PAS method. These included 15 glands from children, and 13 glands from pregnant women. The pituitaries were fixed in 10 per cent neutral formol saline and sectioned at a thickness of 4 micra in the horizontal plane. Adjacent sections were stained by hematoxylin and eosin and the Mann stain. A few selected glands were also stained by the Romeis (16) and Gomori (17) techniques.

Iron-PAS staining technique (modified from Ritter-Oleson) (15)

The details of the technique are as follows:

1. Decerate sections through xylol and hydrate through alcohols to water.
2. Treat for ten minutes in equal parts of dialyzed iron and 2 *M* acetic acid.
3. Wash well in distilled water.
4. Treat for five minutes in equal parts of 0.02 *M* potassium ferrocyanide and 0.14 *M* hydrochloric acid.
5. Wash well in distilled water.
6. Oxidize for five minutes in 0.5 per cent aqueous periodic acid.
7. Rinse in distilled water.
8. Treat for fifteen minutes in Schiff's reagent. This should be renewed frequently. For best results change every seven days or every 50 sections.
9. Rinse for three minutes in each of three changes of sulphurous acid rinses.
10. Wash under running tap water for ten minutes.
11. Stain for one minute in 0.5 per cent aqueous orange G (C.I. 27).
12. Without rinsing, transfer directly to 1.0 per cent aqueous phosphotungstic acid for thirty seconds.
13. Rinse in 1.0 per cent aqueous acetic acid.
14. Dehydrate, clear and mount.

Solutions

Schiff's reagent is prepared in this laboratory according to Lillie's directions: Add 1.0 Gm. of basic fuchsin (C.I. 677) and 1.9 Gm. of sodium metabisulphite to 100 cc. of 0.15 *N* hydrochloric acid. Shake at intervals for two hours. Add 500 mg. of fresh activated charcoal (Norite). Shake for one to two minutes. Filter twice. The solution is ready for immediate use. Store in a refrigerator.

Sulphurous acid rinse, to make 1 liter:

Sodium metabisulphite	4 Gm.
Hydrochloric acid	10 cc.
Distilled water	1,000 cc.

RESULTS

By this staining technique the following cell types were identified (Figs. 1 and 2):

1. *Alpha cells.* These are acidophils. They stain yellow-orange with orange G.

2. *Beta cells.* These are heavily granulated basophils. They stain red with the PAS technique.

3. *Gamma cells.* Because the PAS technique is more discriminative than earlier pituitary staining methods, some cells classified as chromophobes by former procedures have been found to contain granules. We call these lightly PAS-positive cells, gamma cells. Their final color is usually a shade of purple due to combined staining with iron-ferrocyanide and PAS. They are lightly colored because either they are filled with very fine granules or they contain coarser granules sparsely scattered in the cytoplasm.

4. *Delta cells.* These appear as lighter staining basophils with the conventional Mann stain. With the iron-PAS technique they can be distinguished easily from the beta cells by their dark blue-purple color,[1] their smaller size and oval shape. They are scattered singly throughout the

FIG. 1. Anterior pituitary in a case of *sudden death* from cardiac arrest during valvulotomy (A-316/56, female aged 49). Alpha, beta and delta cells and chromophobes are seen. The contrast between the red beta cell and the blue delta cell was accentuated by the use of a green filter. Iron-PAS stain. ×1820.

[1] The result of combined staining with iron-ferrocyanide and PAS.

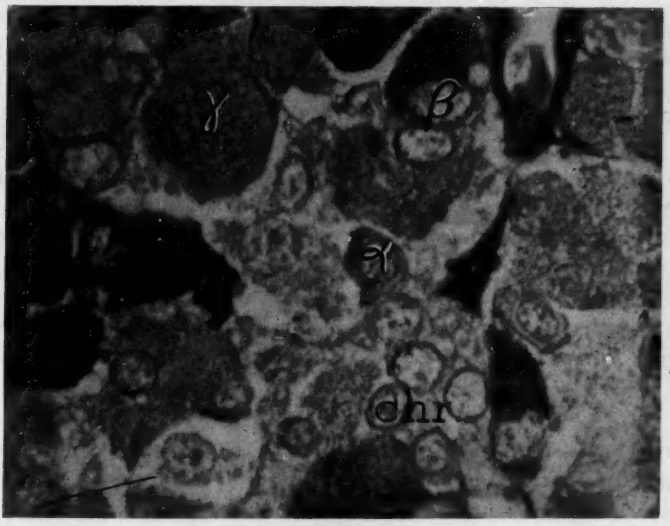

FIG. 2. Anterior pituitary in a case of *short acute illness* due to a berry aneurysm which ruptured seven days before death (A-399/56, female aged 52). There are many gamma cells. Alpha and beta cells, and chromophobes, are also present, but no delta cells are seen. Iron-PAS stain. ×960.

gland. At least a few delta cells were found in every adult gland examined, but their number varied more than that of the other cell types. No delta cells were found in the pituitaries of children and pregnant women.

5. *Chromophobes.* This term is reserved for cells without obvious granulation.

Neighboring sections of several glands containing a varying number of delta cells were stained by the iron-PAS technique, by Romeis' resorcin-fuchsin method (16), by Gomori's aldehyde fuchsin procedure (17) and by the PAS-orange G method used by Russfield (9, 18).

Beta and delta cells could be easily recognized by their characteristic shape and distribution. Beta cells are large polyhedral cells usually found in localized aggregations, more abundant in the antero-medial region of the gland. Their relative number does not vary greatly. In contrast, the delta cells are usually smaller, oval in shape and are scattered singly throughout the whole gland. Their number is quite variable between glands, so that by comparing sections containing different proportions of delta cells, these cells could be positively identified with each of the 4 stains.

In agreement with Romeis (19), beta cells were stained brown-violet to blue-violet with resorcin-fuchsin, whereas delta cells were colored a bright cobalt-blue. Excellent contrast between these 2 cell types was achieved.

However, the vivid red color of the alpha cells resulted in an overall picture which was not as satisfactory as that obtained by the iron-PAS technique.

The application of Gomori's aldehyde-fuchsin procedure, which utilizes iodine as an oxidizing agent and light green as a counterstain, resulted in purple beta cells. This reaction indicates a strong affinity for aldehyde fuchsin. The delta cells took up the aldehyde-fuchsin stain only slightly and reacted positively to the light green counterstain, which gave them a blue-green color. This color contrast is similar to that described by Halmi for the pituitary of the rat (20) and by Russell (21) for the human gland.

With PAS-orange G, the beta cells were stained brick-red, and the gamma cells light mauve or pink. Without a counterstain it was difficult to distinguish between beta cells and heavily granulated delta cells.

DISCUSSION

There is still some difference of opinion regarding the classification of cell types found in the human pituitary gland. Table 1 has been modified from Russfield's presentation (9) to show what we believe is the relationship of our beta, delta and gamma cells to the cells described by other authors.

Crooke and Russell (22), using a Mallory-type technique, which stains acidophils red and basophils blue, described "transitional basophils" in a series of normal pituitary glands. These cells differed from normal basophils in the density and color of their granules. The more heavily granulated of these transitional basophils may correspond to our delta cells, and the lighter ones to our gamma cells.

Romeis (19), using a resorcin-fuchsin technique, described the beta cell as the well recognized abundant cyanophil and the delta cell as a separate type of basophil which was scarcer and less constant in number. By applying the same method to our sections, we established that the beta and delta cells of Romeis were identical with our beta and delta cells respectively.

Mellgren (11) did not recognize the delta cell as a separate entity, but referred to sparsely granulated basophils, which probably correspond to both our delta and gamma cells.

Pearse (3–5) applied the PAS stain to the human hypophysis. He referred to all PAS-positive cells as members of the "mucoid" series. The heavily granulated "maximal" mucoid cells correspond to the standard beta cells; but some of the mucoid cells contain PAS-positive granules with staining properties different from those in the beta cells. Darkly staining cells of this latter type correspond to our delta cells. We would classify as gamma cells the more lightly granulated, disperse, punctate and vesiculated mucoid cells described by Pearse.

The lightly granulated PAS-positive cells were called amphophils by

TABLE 1. COMPARISON OF VARIOUS CLASSIFICATIONS OF CELLS FOUND IN THE NORMAL HUMAN ADENOHYPOPHYSIS

Author	Stain	Cell types				
This report	*Ritter and Oleson: Iron-PAS*	*alpha (orange)*	*beta (red)*	*delta (blue)*	*gamma (light blue)*	*chromophobe*
Crooke & Russell (22)	Mallory: Acid fuchsin-aniline blue	acidophil (red)	basophil (blue-purple)	transitional basophil (blue)	transitional basophil (light blue)	chromophobe
Romeis (19)	Resorcin-fuchsin	alpha (red)	beta (brown-violet)	delta (cobalt-blue)	gamma (light purple)	chromophobe
Mellgren (11)	Hematoxylin-Magdelene red	acidophil (red)	basophil (purple)	sparsely granulated basophil (light purple)	sparsely granulated basophil (light purple)	chromophobe
Pearse (3–5)	Trichrome-PAS	acidophil (orange)	maximal mucoid with beta granules (red)	maximal or intermediate mucoid with gamma granules (lighter red)	intermediate mucoid, diffuse, punctate, vesiculate (pink)	chromophobe
Russfield (9); Burt & Velardo (18)	PAS-orange G	acidophil (orange)	basophil (red)	amphophil (deep rose)	amphophil (pale pink)	chromophobe
Wilson & Ezrin (14)	PAS-methyl blue	alpha (orange)	PAS-red (red)	PAS-purple (dark blue-purple)		chromophobe
Russell (21)	Iodine-aldehyde-fuchsin; Light green-orange G	acidophil (orange)	beta (purple)	delta (green)		chromophobe
Adams & Swettenham (25)	Performic acid-alcian blue; PAS-orange G	acidophil (orange)	R-granules (red-purple)	S-granules (blue)		chromophobe
Adams & Swettenham (25)	Performic acid-aldehyde fuchsin; Light green-orange G	acidophil (orange)	R-granules (green)	S-granules (purple)		chromophobe

Burt (Russfield) (18, 9) because she noted, as did Mellgren, that under certain conditions they were stainable with both acid and basic dyes. By applying the PAS-orange G technique (which Russfield used) to our sections, no clear differentiation between the various PAS-positive cells was obtained.

However, as was shown in an earlier report (14), when methyl blue was added to the PAS-orange G technique a sharp distinction between PAS-red beta cells and PAS-blue-purple delta cells was achieved. Since this method required visual control for consistent staining, a more reliable procedure was sought. The iron-PAS method of Ritter and Oleson was found to provide satisfactory contrast between red and blue-purple colored granules when it was performed as a routine procedure without visual control.

Russell (21) applied Gomori's aldehyde-fuchsin technique to the human pituitary and obtained staining reactions similar to those described by Halmi in the rat (20). Beta cells were strongly aldehyde-fuchsin positive and were colored purple, whereas delta cells were less reactive to the aldehyde-fuchsin but took up the light green counterstain, which resulted in a blue-green tint. We obtained similar results with this technique applied to sections of human pituitaries of the present series as well as to some rat pituitaries. Halmi believed that his green delta cell was equivalent to the "gonadotroph" of Purves and Griesbach (23, 24), and Russell suggested that this might also be the case in the human, although she admitted that direct evidence was lacking. Russfield (9), in an excellent review of the cytology of the adenohypophysis, stated that the beta cell of Romeis corresponds morphologically to the human amphophil, and the delta cell to the ordinary, well granulated basophil. In our view it is the beta cell which corresponds morphologically and also in its anatomic distribution in the gland to the common, well granulated basophil. We believe Russfield has included Romeis' delta and gamma cells in her amphophil group.

Recently Adams and Swettenham (25) published histochemical evidence in support of the existence of 2 types of basophil in the human adenohypophysis. They described a cystine-containing granule (S-granule) which was found mainly in the delta cells, and an R-granule with different histochemical reactions found mainly in the beta cells. These authors also used Gomori's aldehyde-fuchsin technique (17), but changed the oxidizing agent from iodine to performic acid. This resulted in a differentiation between the 2 types of basophil in which the beta cells stained green and the delta cells purple. These colors are the reverse of those obtained by Halmi (20) and Russell (21) after iodine oxidation.

The resorcin-fuchsin, aldehyde-fuchsin, PAS-methyl blue and iron-PAS procedures all show that in the human pituitary gland the delta cell can be recognized as a distinctively colored cell, using a variety of staining meth-

ods. It would seem justifiable to consider the delta cell as a separate cell type, not only because it stains differently from the beta cells but also because, as will be seen, it behaves differently under certain physiologic and pathologic circumstances.

Part B. Effect of the duration of illness on the proportion of delta cells

MATERIAL AND METHODS

Counting techniques

Sixty-six well preserved pituitaries obtained from routine autopsy cases at the Toronto General Hospital, and 5 specimens from cases of accidental sudden death outside the hospital[2] were selected for counting. All cases known to have had endocrine disease, or to have received endocrine therapy were excluded. The following 3 groups of histologic sections were included in the study:

1. Those in which, on preliminary inspection, there were many delta cells.
2. Those from patients who to our knowledge were in good health until twenty-four hours before death.
3. Those from a random group of patients who died of an illness of longer than twenty-four hours' duration.

In order to eliminate subjective prejudice, each slide was drawn at random from the group of selected sections and the cells counted without the operator's knowledge of the case history.

The counts were made of the cells in a horizontal section from what was estimated to be the center of the gland.[3] Under oil immersion, by a modification of the technique of Rasmussen and Herrick (26) the cells in fields 1 millimeter apart were counted; this distance was equivalent to about 6 fields. Two to three thousand cells per section were classified as one or other of the 5 types described in Part A. A blue filter (daylight type) increased the contrast between red and blue-purple cells.

RESULTS

The results of the cell counts were classified in 2 ways—clinically and histologically.

1. *Clinical*

The cases were divided into 3 groups according to the estimated length of the terminal illness:

(a) Sudden death (in less than twenty-four hours)
(b) Short illness (twenty-four hours to fourteen days)
(c) Long illness (more than two weeks).

The percentage of the various cell types in these 3 groups is recorded in Table 2. Alpha and beta cells were remarkably constant in all 3 groups.

[2] Obtained through the courtesy of Dr. Frederick Jaffe, Regional Pathologist, Attorney General's Department, Province of Ontario.

[3] A single horizontal section is more representative of the cell population than a single section taken in any other plane (26, 27).

TABLE 2. CORRELATION OF PITUITARY CELL COUNTS AND DURATION OF ILLNESS

Duration of illness	No. of cases	Proportion of cell types				
		Delta %	Gamma %	Alpha %	Beta %	Chromophobe %
Sudden death (in less than 24 hrs.)	23	8.46±0.8*	7.41±0.8	49.00±2.7	13.46±1.2	21.40±2.6
Short illness (1 to 15 days)	25	4.56±0.7	8.12±0.6	50.53±2.5	12.54±1.5	23.80±2.6
Long illness (more than 2 weeks)	23	1.93±0.4	9.46±0.8	51.39±2.6	13.03±1.4	23.92±2.3

* Standard error.

Statistical analysis

P values (less than 0.05)

	Sudden *vs.* Short	Sudden *vs.* Long	Short *vs.* Long
Ratio Delta/Gamma	0.01	0.001	0.01
Delta %	0.001	0.001	0.01

Chromophobes were slightly increased in longer illnesses, but the change was not significant. *The delta cells were the most labile cell type. They decreased from 8.46 ±0.8 per cent in the "sudden death" group, to 4.56 ±0.7 per cent after a short acute illness, to 1.93 ±0.4 per cent following prolonged illness.* The relative decrease in delta cells with increasing duration of illness is shown graphically in the upper half of Figure 3.

The presence of transitional forms between delta and gamma cells suggests that at least some gamma cells develop from the degranulation of delta cells. Indeed, there was a progressive increase in gamma cells from 7.41 ±0.8 per cent, to 8.12 ±0.6 per cent, to 9.46 ±0.8 per cent in the sudden, short, and long illness groups respectively, but these differences were not significant.

A useful index is obtained by calculating the ratio of delta to gamma cells. There were about 1½ times as many delta as gamma cells in cases of sudden death (Table 3). This ratio decreased significantly to 2/3 in short illness (Table 4), and to 1/4 in prolonged disease (Table 5).

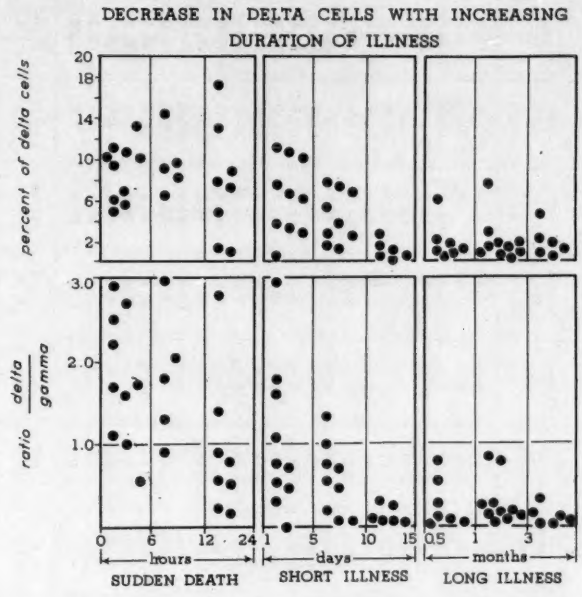

FIG. 3. Note that none of the patients surviving longer than ten days had more delta than gamma cells.

2. *Histologic*

The same results were expressed in a different way by grouping counts according to whether either delta cells or gamma cells were present in greater number.

Table 6 and the lower half of Figure 3 show that the pituitaries with more delta than gamma cells (D/G>1.0) all came from patients who had been sick less than ten days. Sixteen out of 22 (or 73 per cent) of these were obtained from persons who died less than twenty-four hours after the onset of their final illness or injury. On the other hand, of the sections in which there were more gamma cells (D/G <1.0), only 7 out of the 49 (or 14 per cent) came from the "sudden death" group.

Although age and sex separately did not have a significant influence on the weight of the pituitary, postmenopausal women as a group had significantly heavier pituitaries than did all the other subjects. This is a generally accepted observation (28). The greater weight of the pituitaries in the long-illness category, although it appeared significant, may have been partly due to the greater proportion of postmenopausal women in this group. Cell counts were not influenced by the slight disproportion in age and weight distribution in the various groups.

TABLE 3. PITUITARY CELL COUNTS IN CASES OF SUDDEN DEATH

Necropsy No.	Patient		Illness	Approx. duration (hours)	Wt. of pituitary (Gm.)	Ratio delta/ gamma	Delta %	Gamma %	Alpha %	Beta %	Chromophobe %
	Age (yrs.)	Sex									
126/56	23	F	Cardiac arrest during pericardiectomy	<1	0.46	1.53	6.6	4.3	47.0	30.0	12.0
118/56	30	F	Ruptured aneurysm	1	0.37	2.74	5.5	2.0	73.6	13.9	4.5
160/57	60	M	Dissecting aneurysm	1	0.76	2.50	6.0	2.4	63.0	19.6	9.0
297/57	28	F	Hemorrhage during valvulotomy	2–3	0.80	2.21	10.0	4.5	57.0	6.4	21.6
381/57	55	F	Cerebral hemorrhage	5	0.61	1.69	9.5	5.6	65.6	12.3	5.7
266/57	45	M	Cardiac arrest during septal repair	5	0.66	2.84	11.1	3.9	30.7	16.6	37.9
316/56	49	F	Cardiac arrest during valvulotomy	5	0.64	0.49	4.8	9.7	65.0	13.4	7.1
165/57	65	M	Cardiac arrest during hernial repair	5	0.70	1.13	10.9	9.6	46.0	15.9	17.6
404/56	72	M	Acute pulmonary edema	5	0.41	1.62	12.8	7.9	44.1	10.7	24.1
P.27	54	F	Head injury	6	0.60	1.02	10.0	9.8	56.7	12.9	10.1
273/56	46	M	Myocardial infarction	8	0.66	2.00	9.4	4.7	55.7	15.4	14.7
315/57	74	M	Myocardial infarction	12	0.47	1.24	8.1	6.5	52.5	11.4	21.4
P.38	41	M	Crushed chest	12	0.63	1.78	8.9	5.0	37.8	16.6	31.3
P.36	61	M	Crushed chest	12	0.43	0.90	6.4	7.1	49.0	16.8	20.3
P.35	27	M	Multiple injuries	12	—	3.12	14.1	4.5	55.5	2.2	23.6
P.25	17	M	Crushed chest	12	0.66	0.10	0.8	7.9	45.1	20.8	24.7
71/56	42	F	Cerebral hemorrhage	17	0.69	0.68	8.2	12.0	30.0	20.3	29.2
206/57	67	F	Subarachnoid hemorrhage	24	0.63	0.45	7.9	17.5	27.6	6.4	41.0
355/56	69	M	Myocardial infarction	24	0.52	1.40	17.4	12.4	41.4	11.4	18.5
401/56	74	F	Myocardial infarction	24	0.57	0.95	7.1	7.5	29.0	14.6	38.8
421/56	33	M	Hemorrhage following pericardiectomy	24	0.37	2.80	12.9	4.6	48.4	7.5	26.7
124/57	72	F	Hemorrhage following cholecystectomy	24	—	0.64	4.8	7.5	64.9	6.5	16.2
90/57	65	F	Myocardial infarction	24	0.87	0.09	1.2	13.5	41.2	7.8	36.4
Average (23 cases)					0.59 ± .03	1.48* ±0.19	8.46 ±0.8	7.41 ±0.8	49.00 ±2.7	13.46 ±1.2	21.40 ±2.6

* This figure represents the average of the ratios rather than the ratio of the averages.

TABLE 4. PITUITARY CELL COUNTS IN CASES OF SHORT ILLNESS

Necropsy No.	Patient		Illness	Approx. duration (days)	Wt. of pituitary (Gm.)	Ratio delta/ gamma	Delta %	Gamma %	Alpha %	Beta %	Chromophobe %
	Age (yrs.)	Sex									
227/57	15	M	Cerebral hemorrhage	2	0.55	0.74	2.5	3.4	48.6	5.3	39.1
235/57	57	M	Postoperative shock	3	0.58	0.55	3.7	6.7	51.0	5.2	33.1
233/57	90	F	Fracture of femur	3	0.71	0.03	0.3	10.4	44.6	16.7	27.9
125/57	65	M	Hemorrhage after repair of carotid artery	3	0.78	1.79	11.2	6.2	44.9	6.3	30.3
259/57	39	M	Aortic stenosis	3	0.66	3.21	6.1	1.9	66.0	17.5	8.2
240/57	65	F	Pneumonia	3	0.52	0.30	3.1	10.5	57.8	14.2	14.4
166/57	49	M	Subarchnoid hemorrhage	3	0.44	0.45	6.3	13.9	48.2	6.8	24.1
275/57	54	F	Myocardial infarction	5	0.60	1.14	10.0	8.8	40.3	5.2	35.4
523/56	83	M	Myocardial infarction	5	0.42	0.75	7.5	9.7	53.2	10.7	19.2
125/56	49	M	Myocardial infarction	5	0.70	1.58	10.6	6.7	55.7	4.2	22.8
131/57	48	F	Ruptured berry aneurysm	6	0.46	0.45	3.8	8.7	36.9	17.2	32.9
242/57	68	M	Cerebral hemorrhage	6	0.47	0.56	2.6	4.6	73.0	6.9	12.4
262/57	93	F	Fracture of femur	7	0.60	0.75	5.6	7.5	60.3	13.4	12.7
519/56	73	F	Cerebral softening	7	0.70	0.21	2.4	11.6	40.8	9.3	36.0
521/56	81	F	Cerebral embolism	7	0.42	0.07	1.3	16.6	56.0	5.3	19.0
399/56	52	F	Ruptured berry aneurysm	7	0.67	0.12	1.5	12.1	38.0	23.4	25.3
223/57	81	M	Cerebral hemorrhage	7	0.53	1.02	7.9	7.7	48.6	6.7	28.3
476/56	41	M	Valvulotomy, postop. complications	8	0.58	0.73	6.9	9.4	46.1	15.5	21.9
489/56	70	M	Cardiac tamponade	10	0.78	1.36	7.5	5.5	63.5	11.9	11.1
45/57	55	F	Myocardial infarction	10	0.72	0.27	1.4	5.2	24.9	11.9	56.8
103/57	43	F	Uremia	11	0.72	0.15	0.1	6.5	52.4	34.0	6.7
232/57	58	M	Pulmonary embolism, postop.	12	0.65	0.05	0.3	5.6	73.6	9.2	10.6
228/57	17	M	Brain hemorrhage	13	0.68	0.04	0.3	6.9	59.5	12.0	21.2
38/57	77	F	Myocardial infarction	13	0.58	0.90	9.0	10.0	24.2	30.0	26.8
90/56	54	F	Rheumatic fever, acute	15	0.62	0.33	2.3	7.0	55.2	14.7	20.5
Average (25 cases)					0.60 ± .02	0.69* ±0.15	4.56 ±0.7	8.12 ±0.6	50.53 ±2.5	12.54 ±1.5	23.80 ±2.6

* This figure represents the average of the ratios rather than the ratio of the averages.

TABLE 5. PITUITARY CELL COUNTS IN CASES OF LONG ILLNESS

Necropsy No.	Patient		Illness	Approx. duration	Wt. of pituitary (Gm.)	Ratio delta/ gamma	Delta %	Gamma %	Alpha %	Beta %	Chromophobe %
	Age (yrs.)	Sex									
239/57	68	M	Carcinoma of parotid gland	3 wks.	0.55	0.60	6.3	10.5	50.7	10.4	21.9
139/57	36	M	Mitral stenosis	3 wks.	0.57	0.26	2.1	7.9	54.1	13.9	21.8
68/57	74	F	Bronchopneumonia	3 wks.	0.60	0.09	0.8	8.1	54.2	19.2	17.2
237/57	77	F	Carcinoma of gall bladder	4 wks.	0.78	0.06	0.6	10.0	45.8	15.5	27.7
219/57	61	F	Carcinomatosis	4 wks.	0.72	0.05	0.5	9.3	66.2	8.8	15.6
214/57	68	F	Ruptured berry aneurysm	4 wks.	0.64	0.13	1.2	9.3	51.5	11.7	26.4
210/57	53	M	Cardiac failure	4 wks.	0.63	0.27	1.8	6.5	71.8	2.1	17.4
174/57	33	F	Purulent pericarditis	6 wks.	0.50	0.10	0.8	7.7	55.5	7.9	28.2
375/56	78	F	Myocardial infarction	6 wks.	0.89	0.19	2.3	18.3	41.4	12.1	24.4
253/57	57	M	Carcinomatosis	6 wks.	0.57	0.79	3.1	3.9	38.8	15.6	37.7
96/57	71	M	Cerebral infarction	6 wks.	0.56	0.15	0.7	4.6	59.5	24.9	10.1
218/57	56	M	Chronic bronchitis and emphysema	2 mos.	0.76	0.78	7.8	10.0	64.0	6.7	10.8
167/57	55	F	Peritonitis	2 mos.	0.86	0.13	1.4	10.8	65.0	8.9	14.2
213/57	37	F	Cerebral infarction	2 mos.	0.62	0.26	0.2	7.7	47.0	8.7	35.5
249/57	77	F	Retroperitoneal sarcoma	2 mos.	0.74	0.19	1.6	8.4	64.5	5.2	19.1
164/57	64	F	Uremia & bronchopneumonia	3 mos.	0.74	0.06	0.9	14.0	41.2	17.0	27.0
124/56	66	M	Carcinoma of epidermis	3 mos.	0.72	0.15	1.7	10.7	41.0	24.3	22.5
226/57	27	F	Chronic rheumatic heart disease	6 mos.	0.69	0.10	0.6	5.7	36.7	16.0	41.0
196/57	65	M	Carcinomatosis & subdural hemorrhage (4 days)	6 mos.	0.59	0.14	1.9	13.5	62.5	10.6	11.1
78/56	44	F	Carcinoma of esophagus	6 mos.	0.75	0.10	1.0	9.7	66.0	6.1	17.0
241/57	75	F	Carcinoma of breast	1 yr.	0.82	0.36	4.5	12.5	42.0	8.7	32.2
493/56	76	F	Carcinoma of uterus	1 yr.	0.80	0.13	2.0	15.4	41.5	19.4	23.5
402/56	72	M	Carcinoma of prostate	2 yrs.	0.72	0.16	0.5	3.0	21.1	26.1	47.9
Average (23 cases)					0.69 ± .02	0.23* ±0.04	1.93 ±0.4	9.46 ±0.8	51.39 ±2.6	13.03 ±1.4	23.92 ±2.3

* This figure represents the average of the ratios rather than the ratio of the averages.

TABLE 6. DECREASE IN RATIO OF DELTA/GAMMA CELLS WITH INCREASING DURATION OF ILLNESS*

Duration of illness	Delta cell group, delta:gamma>1.0		Gamma cell group, delta:gamma <1.0	
	Cumulative cases		Cumulative cases	
	Number	%	Number	%
Under 12 hrs.	13	59	3	6
Under 1 day	16	73	7	14
Under 2 days			8	16
Under 3 days	18	82	12	24
Under 5 days	20	91	13	26
Under 7 days	21	95	19	39
Under 10 days	22	100	22	45
Under 14 days			26	53
Under 21 days			29	59
Under 28 days			33	67
Over 28 days			49	100

* None of the patients surviving more than ten days had more delta than gamma cells.

DISCUSSION

The closest approximation to a normal pituitary gland is a specimen obtained shortly after the instantaneous death of a healthy person. We were fortunate in obtaining 5 such glands suitable for counting, from medicolegal cases. All of these contained an abundance of delta cells. The pituitaries from patients who died in the hospital within twenty-four hours of the onset of an acute illness also contained many delta cells and were, therefore, included in the same group. If the illness lasted longer than twenty-four hours, the proportion of delta cells gradually decreased. The pituitary appears to be a dynamic gland which in the normal adult contains approximately 8.5 per cent delta cells.

The ideal statistical approach to the study of human material is to work with sufficiently large numbers to mask all variables except the one singled out for study. In this project the only consistent difference between the 3 groups was the duration of the terminal illness. This grouping, with time as the common factor, showed that with increasing duration of illness there was a significant change in the proportion of delta cells. The length of illness itself, however, is not a single entity, but represents a complex of hormonal and metabolic changes. Which of these changes are reflected in the decrease of delta cells is a matter that is open to speculation. It is felt that

the apparent disappearance of delta cells represents a process of degranulation to a form which is no longer recognizable as a distinct cell type. This degranulation may indicate increased hormonal output, which may or may not continue when the cell is degranulated.

Since it is known that there is an increased secretion of adrenocortical hormones during disease (29), the most obvious interpretation is that delta cells contain a store of readily available ACTH or precursor, which is released when there is more need for this hormone. Currie and Symington (30) correlated histologic changes in the pituitary with those in the adrenal cortex. They found that degranulation of "maximal mucoid cells" was usually accompanied by depletion of adrenal lipid. These signs of activity were most pronounced in patients who died after an acute illness of less than one week's duration. If the final illness lasted longer than six weeks, both glands reverted to a normal histologic pattern. Rogers and Williams (31) reported that, irrespective of the cause of death, patients who died suddenly had smaller adrenals than those whose illness was prolonged.

Time relations are crucial in the interpretation of our findings. The degranulation of the delta cells appears to take place gradually over several days. Although, at present, physiologic amounts of ACTH cannot be measured in systemic venous blood (32), there is good indirect evidence that ACTH is released very rapidly even after a fairly minor "stress." Moore (29) showed that there may be a two- to threefold rise in the concentration of serum 17-hydroxycorticoids within thirty minutes of induction of ether anesthesia, and a further increase to four or five times the resting level during surgery. In an uncomplicated postoperative course, the level declined within four to eight hours and was often normal by the next morning, but remained elevated if there was infection.

The delta cells appear to lose their granules more slowly than would be expected if the loss of granulation were due to increased secretion of ACTH in response to stress. It may be that only part of the available store of ACTH is liberated immediately, and that the rest is released gradually, or that new synthesis of the hormone is replenishing the cells with granules as fast as they are depleted.

Another interpretation of the function of the delta cells, more directly related to their positive reaction to the PAS stain, is that they make one or more gonadotropins. The time relations of the degranulation of the delta cells corresponds quite closely to the pattern of secretion of gonadotropins suggested by Sohval *et al.* (33) in a study of urinary gonadotropins following surgery. In 8 out of 18 patients there was an increased excretion of gonadotropins after operation. In all 8 cases the rise occurred within four days, and in 4 of the cases the urinary gonadotropins increased as early as the first day. The duration of the increase was variable. In 1 patient the

gonadotropin excretion dropped sharply as early as twelve hours postoperatively, whereas in some others it remained elevated for seven to eight days. The gradual loss of granules in the delta cells may indicate a release of stored gonadotropins, which is not followed by synthesis and further storage. The excretion of pituitary gonadotropins in the urine is usually decreased in chronic wasting disease. The scarcity of delta cells in these cases may reflect decreased production of gonadotropins in the pituitary. Burt (Russfield) and Velardo (18) reported the results of assay of stored gonadotropins in a series of human pituitary glands. They found that pituitaries from patients dying of cancer contained little or no stored gonadotropins. In patients dying of cerebrovascular and cardiovascular accidents, appreciable quantiti of gonadotropins were found. Although the duration of the final illness was not stated, it may be inferred that patients in the latter group did not survive long after the onset of their vascular accidents. In counting cells, Burt and Velardo did not distinguish delta cells from beta cells and amphophils. In their study, if the pituitaries from cases of sudden death contained many delta cells, there would be more evidence for gonadotropins originating in the delta cells.

Additional evidence in favor of this hypothesis is the absence of delta cells in the pituitary glands of children and pregnant women. The pituitary gland of the child secretes little or no gonadotropin (34–36). During pregnancy the production of pituitary gonadotropins is inhibited by the large output of estrogens (6, 37).

Herlant (38) and Purves and Griesbach (39) contend that the human delta cell secretes thyrotropin, because Herlant noted an increased number of what was called hypertrophied delta cells in the pituitaries of patients with myxedema. In our opinion gamma cells, which resemble hypertrophied delta cells, may arise from degranulation of either beta or delta cells. We believe that what Herlant saw were gamma cells derived from beta cells. Russell (21) reports that with Gomori's aldehyde-fuchsin technique there is a beta-type reaction from the hypertrophied cells which are abundant in the pituitaries of cases of myedema. From a study of endocrine disturbances that will be presented in detail in another paper, we have concluded that gamma cells are degranulated, actively secreting forms of beta and delta cells, and sometimes even of alpha cells. On this point we agree with Russfield's (9) interpretation of the function of the amphophils. Objection might be raised to classifying a group of cells of heterogeneous origin and function as a separate cell type, yet with the iron-PAS technique and with most other stains it is not possible to distinguish between gamma cells of various origins; therefore, in differential cell counts we compromised by putting all gamma cells into a single category.

Ancillary evidence from other species supports the hypothesis that the

delta cell secretes gonadotropin. We have referred to the work of Halmi (20) and Purves and Griesbach (23, 24) in the rat. Recently Saxén *et al.* (40), using the PAS-methyl blue technique, identified 2 types of PAS-positive cells in the hypophysis of the adult frog, and related the blue-purple (delta) cell with gonadotropic activity.

Whatever the function of the delta cells may be, it can be said that these cells are normal components of the adult human pituitary, and that their number gradually diminishes in disease. In view of the absence of these cells in childhood and pregnancy, it is our opinion that they are the source of one or more of the pituitary gonadotropins.

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ADRENOCORTICAL CARCINOMA IN MAN. THE EFFECT OF AMPHENONE ON INDIVIDUAL KETOSTEROIDS*

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ABSTRACT

Five patients with adrenocortical carcinoma were studied with respect to the production of various individual 17-ketosteroids during control periods and during treatment with amphenone (3,3-bis(*p*-aminophenyl)butanone-2). In all instances the drug caused a decrease in some or all of the steroid hormone metabolites. A detailed discussion of each major component of the 17-ketosteroids leads to the conclusion that amphenone invariably lowers the production of hydrocortisone but exerts a variable influence upon other components of adrenal secretion. The results support the conclusion that the biosynthesis of adrenocortical hormones can be differentially altered, even in a neoplastic tissue.

THE steroid hormones secreted by the human adrenal eventually appear in the urine as 2 chemical classes of metabolites. Hormones such as hydrocortisone are converted to metabolites which retain an atom of oxygen at carbon 11 of the steroid nucleus; other adrenal hormones which initially lack the C-11 oxygen appear in urine as 11-deoxysteroids—among others, androsterone and etiocholanolone. Metastatic carcinoma of the adrenal cortex in man is invariably accompanied by a marked increase in the production and secretion of both classes of these steroid hormones, although often a great disparity in the relative proportion of either component can be demonstrated with suitable analytical methods. The purpose of this report is to describe steroid isolation studies in 5 patients with adrenocortical carcinoma during control periods and while being treated with amphenone.[1] The principal focus is on the alteration in production of the 2 classes of metabolites that may be achieved with this agent. It is hoped that the results reported will clarify the interpretation of previous data

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[1] Amphenone is 3,3-bis(*p*-aminophenyl)butanone-2 (*cf.* W. L. Benczl and M. J. Allen: Structure of amphenone B and related amphenones, *J. Org. Chem.* **22**: 352, 1957). The compound for these studies was graciously furnished by Dr. Roy Hertz of the National Cancer Institute, Bethesda, Maryland.

and will direct attention to the need for application of more searching analytical methods in the evaluation of similar potential chemotherapeutic agents.

Peterson, Hertz and Lubs (1) and Thorn and associates (2) described marked decreases during amphenone treatment in the excretion of "urinary corticoids," which are presumed to be derived from hydrocortisone. The same authors reported "unimpressive" changes or no change in the urinary 17-ketosteroids. Both groups commented that alterations in the 17-ketosteroids might have been detected had more exact methods of separation been employed. It was previously demonstrated (3, 4) that administration of amphenone to a patient with widely metastatic adrenocortical carcinoma produced a highly significant fall in all the chromatographically isolated steroids which together constitute the "urinary 17-ketosteroids." In view of these apparently conflicting findings and because of the import for a clearer knowledge of secretory function of neoplastic adrenals, detailed consideration is given to the effect of amphenone on the major individual components of the "urinary 17-ketosteroids."

SUMMARY OF CASES

S-52 (study made possible through the cooperation of Drs. Olof Pearson, Leon Hellman, C. D. West and Mortimer Lipsett of this Institute). The patient, a 52-year-old white woman, was admitted to Memorial Center for the first time in June 1956, for study and evaluation. Signs and symptoms of Cushing's syndrome had developed in May 1954, and in April 1955 an adrencortical carcinoma had been removed surgically at another hospital. Postoperatively, there had been a striking clinical remission lasting until February 1956. On admission to Memorial Center in June, the classic clinical and chemical findings of Cushing's syndrome were present, and there was also evidence of multiple pulmonary metastases.

While the patient was in the hospital, a continuous metabolic balance regimen was maintained and 24-hour urine collections were made for steroid analysis. Control and treatment periods were alternated. Treatment periods were as follows:

July 3– 5: Oral hydrocortisone, 150 mg. per day.
July 12–17: Intramuscular ACTH-gel, 240 units per day.
July 26–28: Oral amphenone, 3 Gm. per day. Results of steroid isolation studies during this period are shown in Figure 1.

At the conclusion of these studies, the patient was discharged. No specific medication was recommended. Her clinical status was essentially the same as on admission.

In September 1956, the patient was re-admitted to Memorial Center for the second and the last time. In the interval since discharge, the signs and symptoms of Cushing's syndrome had increased, and signs of congestive heart failure had appeared. Complete metabolic balance studies were again started, and 24-hour urine collections were made for steroid analysis. After control studies from September 10 to 15, oral amphenone was begun in a dosage of 3 Gm. per day. The data charted in Figure 2 are the results obtained on the third, fourth and fifth days of this dosage, with the data of 2 prior control periods for comparison. On September 28, the daily dose was raised to 4 Gm. On October

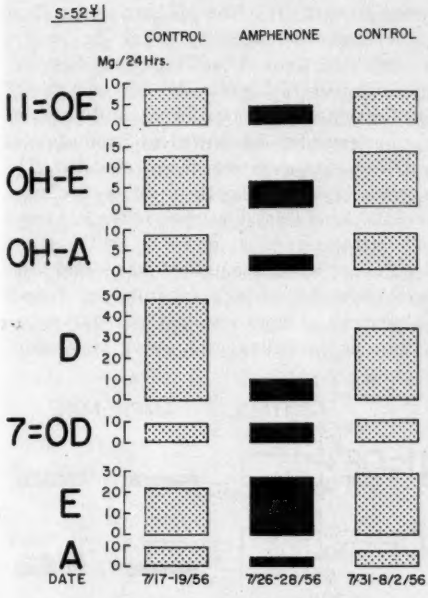

FIG. 1. (Subject S-52.) Comparison of ketosteroid production during treatment with amphenone, and during control periods before and after administration of the drug.

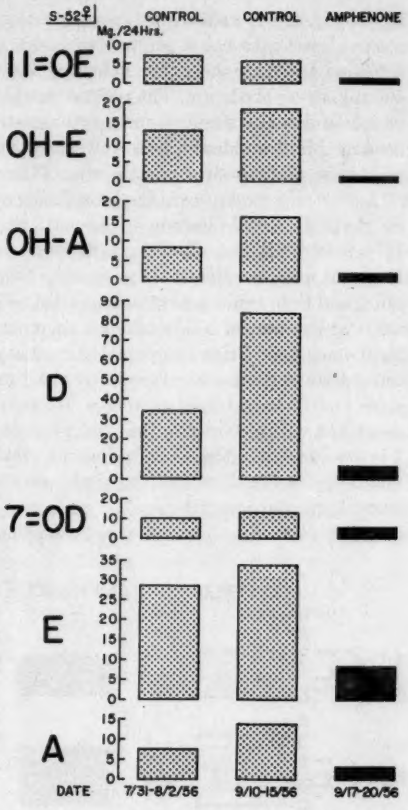

FIG. 2. (Subject S-52.) The change in ketosteroid production with progress of the disease. Compare the effect of amphenone with that shown in Figure 1.

2, amphenone was discontinued because of progressive lethargy. Despite stopping the drug, the patient deteriorated rapidly and died on October 12. Autopsy revealed that the cause of death was cryptococcal meningitis.

G-37 (study made possible through the cooperation of Dr. Sidney C. Werner of Presbyterian Hospital, New York). The patient was a 37-year-old white woman, first seen at Presbyterian Hospital, New York City in January 1957, complaining of amenorrhea of six weeks' duration and bloating of the face for two months. On physical examination, she had the appearance of a woman with Cushing's disease. Facial hair was excessive, and purpura was present over the breasts and abdomen, but there were no striae. The abdomen contained a hard mass in the region of the right kidney. The overlying liver was readily palpable. There was a mild anemia. The serum sodium level was slightly increased (146.3 mEq./L) and the potassium level was slightly decreased (2.4 mEq./L). At operation, a typical carcinoma of the adrenal cortex was found—confirmed

by biopsy. On the third postoperative day, a series of psychotic episodes set in, ending with a lapse into coma for twelve hours. These episodes could not be explained, and subsided spontaneously. On February 20, amphenone was started in an oral dosage of 500 mg. every six hours. The results charted in Figure 3 were obtained during the fourth and fifth days of medication. Approximately two weeks after the last dose, the patient became blind suddenly, but slowly recovered within two weeks. She returned home, where she died about a month later. There was no autopsy.

L-7 (study made possible through the cooperation of Dr. George Thorn, Peter Bent Brigham Hospital, Boston). The patient (previously described by Sobel (5)) was a 7½-year-old girl who was hospitalized on the metabolic ward at the Peter Bent Brigham Hospital until her death three months later. At the age of 7 years she first manifested mild, and then rapidly progressing, signs of virilization. At the age of 7 years and 3 months surgical excision of a left adrenal tumor was performed and followed by irradiation of the tumor bed. Prior to operation, urinary 17-ketosteroid excretion was elevated. The immediate response to excision of the tumor was gratifying, both in terms of clinical signs and 17-ketosteroid excretion. However, a palpable mass developed in the left lower quadrant within two months, and pulmonary metastases became evident at the age of 7 years and 6 months. On admission to the metabolic ward, the patient was found to be markedly virilized, with only slight manifestations of Cushing's syndrome, and moderate hypertension. During the period of observation, there was marked aggravation of signs and symptoms of the endocrine disorder, including progressive virilization

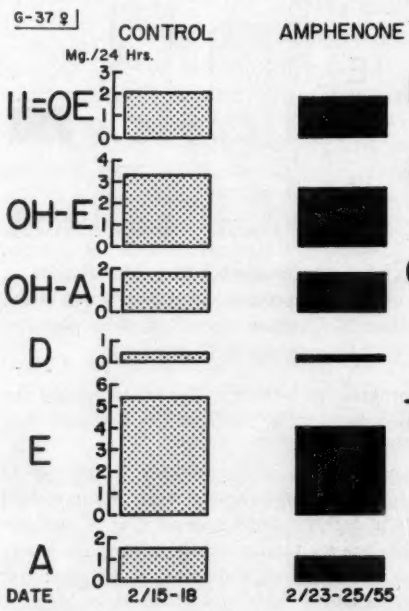

FIG. 3. (Subject G-37.) Comparison of ketosteroid production during treatment with amphenone and during the prior control period.

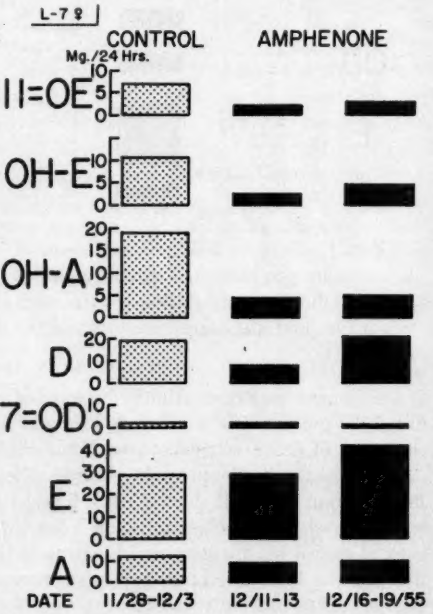

FIG. 4. (Subject L-7.) Comparison of ketosteroid production during two periods of continued treatment with amphenone, and during the prior control period.

hypertension and the development of obvious Cushing's syndrome with a moon face, striae, voracious appetite and glucosuria. Since there had been a suggestive response to adrenocorticotropin (ACTH) shortly after admission, in terms of urinary steroid excretion, the patient underwent hypophysectomy. The clinical course and the changes in urinary steroid excretion suggested a very brief (ten days) decrease in the metabolic activity of the metastatic tumor. Other attempts to control the rapidly progressing malignant lesion, including the administration of amphenone and of N,N′,N″-triethylenethiophosphoramide ("thio-TEPA"), were unsuccessful. Amphenone was begun on December 3, 1955 in dosage of 4 Gm. per day, increased gradually to 9 Gm. per day during the period December 11 to 13. This was the first medication period during which steroid isolation studies were performed. The daily dose was increased gradually to 12 Gm. from December 16 to 19, which was the second period of the study. The results are shown in Figure 4. On the third day after the last course of amphenone therapy, the patient had a generalized convulsion and died despite efforts at resuscitation. Postmortem examination confirmed the presence of a widely metastasizing, relatively undifferentiated, recurring adrenocortical carcinoma.

D-41 (study made possible through the cooperation of Dr. George Thorn, Peter Bent Brigham Hospital, Boston). The clinical description of the patient has been published previously (2). The length of the amphenone period (Fig. 5) was four days, during which the dosage was 3 Gm. per day orally. This was preceded by a day during which a dose of 3 Gm. of amphenone was administered; the urine of this day was not included in the study. Two weeks earlier, 10 Gm. of amphenone had been given during two days.

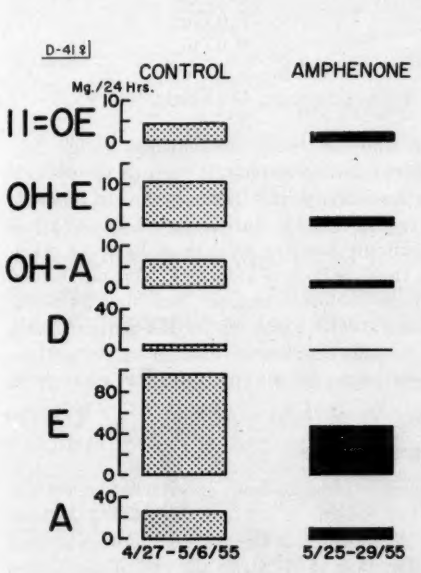

FIG. 5. (Subject D-41.) Comparison of ketosteroid production during administration of amphenone and during the prior control period.

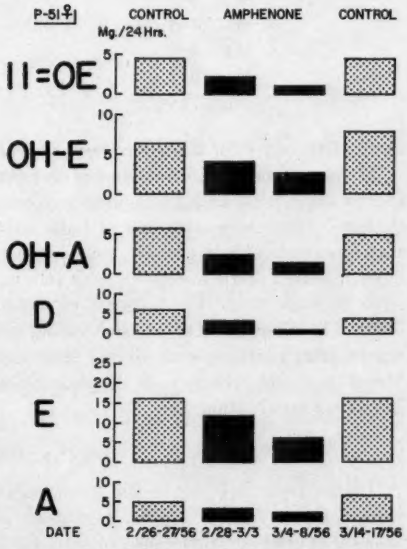

FIG. 6. (Subject P-51.) Comparison of ketosteroid production during two periods of continued treatment with amphenone, and during control periods before and after administration of the drug.

P-51 (study made possible through the cooperation of Drs. Olof Pearson, Leon Hellman, C. D. West and Mortimer Lipsett of this Institute). The patient was a 51-year-old white woman who had experienced gradual onset of weakness and loss of weight beginning in March 1954. This was followed by the development of moon facies, truncal obesity, edema and hypertension. In February 1955, a diagnosis of Cushing's syndrome was made and the patient was referred to Memorial Center for therapy. An adrenocortical carcinoma was removed on April 5, 1955.

The patient improved postoperatively and was discharged. Her condition remained normal until January 23, 1956, when the weakness, moon facies and edema returned. She also complained of pain in the right side of the chest. Physical examination revealed an enlarged liver and hypertension. X-ray examination showed pulmonary metastases, but no definite bony involvement. Urinary 17-ketosteroids were moderately elevated. The glucose tolerance curve was of the diabetic type. The patient was admitted on February 4, 1956, for study during amphenone therapy.

A metabolic balance period was started on February 8, 1956 and was continued throughout the study. She received 9α-fluorohydrocortisone, 10 mg. daily for four days (February 12 to 16); ACTH-gel, 240 units daily for four days (February 19 to 23); and amphenone according to the following schedule:

Feb. 28–29	1.0 Gm.
Feb. 29–Mar. 1	3.0 Gm.
Mar. 1–2	4.5 Gm.
Mar. 2–3	4.0 Gm.
Mar. 3–4	5.0 Gm.
Mar. 4–5	5.0 Gm.
Mar. 5–6	6.0 Gm.
Mar. 6–7	6.0 Gm.
Mar. 7–8	6.0 Gm.

Combined urines for 2 periods were analyzed, and the results are shown in Figure 6.

There was no significant change in potassium, nitrogen, phosphorus or calcium balances during these treatment periods. The sodium and chloride balances showed minimal changes. There was retention of both sodium and chloride during the 9α-fluorohydrocortisone and ACTH periods, reaching a maximum positive balance of 12 to 14 mEq. daily. Sodium and chloride diuresis followed these periods of retention. During amphenone therapy there was a negative sodium and chloride balance, reaching 24 mEq. daily. The creatinuria decreased during treatment with amphenone. The patient had a severe drug reaction with shock, fever and a rash. Amphenone had to be stopped on March 9. Upon recovery, Δ^1-9α-fluorohydrocortisone, 4.0 mg. per day, was given from March 18 to 27, 1956.

ANALYTICAL METHODS

Urines were received in the laboratory as individual 24-hour specimens; collections were complete, as judged by constancy of the creatinine content. Individual 24-hour specimens were combined into suitable pools (as indicated in the charts) and were treated with beef liver β-glucuronidase[2] at pH 5 for five days at 37° C. At the end of incubation

[2] β-Glucuronidase, obtained from the Warner-Chilcott Laboratories, a division of Warner-Lambert Pharmaceutical Company, New York, N. Y. It is available under the trade name Ketodase.

the urine was adjusted to pH 1 and extracted continuously with ether for forty-eight hours. The ether-soluble neutral fraction was separated and the alkali-soluble material was combined with the extracted urine. The urine plus alkali-soluble fraction after neutralization was acidified to 1 *N* with sulfuric acid and re-extracted continuously with ether for forty-eight hours. Again the neutral ether-soluble fraction was separated. Each of these neutral extracts was separately processed into ketonic and nonketonic fractions by means of Girard's reagent T, and the ketonic fractions were further separated into α and β ketosteroid subfractions by means of digitonin. These methods have been described in detail (6). The separate subfractions from each extract were quantitatively chromatographed on paper, using minor modifications of the systems developed by Burton, Zaffaroni and Keutmann (7). The individual steroids eluted from the paper were measured quantitatively by the Zimmermann reaction, according to the method of Talbot, Butler, MacLachlan and Jones (8). The identity of each substance eluted from the chromatograms was confirmed by infrared spectrometry. A number of compounds other than those recorded were identified in all of the patients studied. These will be described elsewhere.

The extracts from Subject L-7 contained a large amount of tarry material, which made processing of the extracts difficult. With this subject, during both control and amphenone periods, it was necessary to re-treat the insoluble tar with Girard's reagent T. The ketonic fractions so obtained were chromatographed separately and the values for the isolated compounds were added in order to obtain the daily steroid production. This difficulty was not encountered with any of the other patients; it may have been due to the large amounts of various drugs with which the patient was treated, or perhaps due to her nearly terminal condition.

RESULTS AND DISCUSSION

11-Hydroxyetiocholanolone (OH-E) and 11-ketoetiocholanolone (11=OE)

These 2 metabolities are derived primarily from hydrocortisone and should be considered together as representative metabolities of that hormone. Quantitatively the greatest production was found in Subject S-52, in whom the amount exceeded 20 mg. per day. Assuming that these 2 compounds account for about 5 to 10 per cent of the metabolities of hydrocortisone,[3] the tumor of this patient was secreting between 200 and 400 mg. of hydrocortisone per twenty-four hours. A reasonable estimate of the daily production of this hormone by normal subjects would be less than 20 mg. per day. It is thus evident that the metastases of this patient produced a great excess of hydrocortisone. The result of this high production was manifest in the extreme symptoms of Cushing's syndrome exhibited by this patient. A comparable situation existed in Subject L-7 whose daily production of the same metabolites during control periods was 18 mg. per twenty-four hours. These 2 patients had the highest excretion of OH-E

[3] This approximation is based on the percentage recovery of these metabolites after the administration of tracer doses of hydrocortisone-4-C^{14} to human subjects. Personal communication of D. K. Fukushima, H. Leon Bradlow and others from these laboratories.

and 11=OE. Among the other 3 patients, the least elevation in 11-oxygenated hormone production (G-37) was a fivefold increase over normal values.

In all of the subjects studied there was a highly significant diminution in the production of these metabolites during administration of amphenone. It is emphasized that this diminution occurred even when the dosage of the drug was 2 grams per day (Subject G-37). There is an important technical factor in connection with the effect of amphenone on these 11-oxygenated metabolites. The extracts of urine obtained during the administration of large quantities of amphenone were contaminated with the drug and its metabolic transformation products. Even though the extracts had been extensively fractionated prior to chromatography, a certain portion of the drug or its metabolites was carried along with the steroids. In the paper chromatograms, a background of brownish material moved into the area where the 11-oxygenated compounds were separated. This background material was not found during control periods without amphenone. Although this material did not interfere with the separation or identification of the steroids studied, it did contribute nonspecific chromogenic material which resulted in a falsely high value for the 11-oxygenated metabolites during the amphenone periods. It was felt that further fractionation in an attempt to remove this background contaminant might result in an excessive loss of steroid and, for this reason, no attempt was made to obtain the steroids in purified form, nor was any correction introduced to take account of the added chromogen. For these reasons it is believed that the estimated values for these 11-oxygenated compounds are maximal and that the true values are probably somewhat lower than those recorded. It was clearly demonstrated that without exception *amphenone caused a significant decrease in the two 11-oxygenated ketosteroids derived from hydrocortisone.*

11-Hydroxyandrosterone (OH-A)

This compound is the major metabolite of another component of the adrenal secretion, 11β-hydroxy-Δ^4-androstene-3,17-dione (9). It is likewise a principal metabolite of adrenosterone, if this substance is produced by the adrenal gland (10). OH-A is a very minor metabolite of hydrocortisone and accounts for less than 2 per cent of the transformation products of that hormone.[3] In contrast with the other two 11-oxygenated ketosteroid metabolites (OH-E and 11=OE), 11-hydroxyandrosterone to a major extent reflects adrenal secretion of a C_{19} 11-oxygenated hormone. It is not surprising, therefore, to find examples among these cases of carcinoma in which the production of this metabolite is disproportionate when compared with 11-hydroxyetiocholanolone and 11-ketoetiocholanolone. Thus Subject L-7, who in control periods excreted 19 mg. of OH-A per twenty-four

hours, produced more of this substance than of the other 2 major 11-oxygenated ketosteroids combined. Under the influence of amphenone, Subject L-7 showed a greater diminution of this metabolite, so that in fact the greatest percentage decrease was demonstrated with OH-A. In Subject G-37, on the other hand, there was no suppression of this component under the influence of a smaller dose of amphenone. In the rest of the subjects *there was a major decrease in the production of the precursors of this metabolite during treatment with amphenone.*

Dehydroisoandrosterone (D)

This compound has been present in the urine of all human subjects thus far examined, whether normal or abnormal. Under most circumstances the amount is relatively small, *i.e.*, 1 mg. or less per day. It was once believed that excessive amounts of this compound are produced in tumors of the adrenal cortex. It is clear from the present series that this is not invariably true. Subject G-37, who had a highly malignant metastatic carcinoma, excreted only 0.4 mg. of D per day during the control period, and the amount was not materially lessened during the period when a small dosage of amphenone was given. Similarly, although excretion was out of the normal range in Subjects D-41 and P-51, both produced less dehydroisoandrosterone than might have been anticipated from the very high values for "total 17-ketosteroids." On the other hand, there was a tremendous production of D by Subjects S-52 and L-7 during control periods. It is apparent that some, but not all, adrenal tumors are capable of excessive production of dehydroisoandrosterone.

All patients, with the exception of G-37, exhibited a pronounced decrease in the production of D during treatment with amphenone. Similarly, in a patient previously reported (3, 4), D production decreased from a level of approximately 175 mg. per day during the control period to approximately 2 mg. per day during administration of amphenone. In general, it appeared to be true in this series of patients that *with higher production of dehydroisoandrosterone during the control period, there was a greater percentage fall during treatment with amphenone.*

7-Ketodehydroisoandrosterone (7 = OD)

This compound,[4] like dehydroisoandrosterone, is a normal urinary constituent and the amount excreted per day is usually small. The substance is

[4] In the report that described the first identification of 7-ketodehydroisoandrosterone from human urine (11), it was stated that although the compound might have been an artifact formed by autoxidation of dehydroisoandrosterone during the isolation procedure, in the lack of direct evidence it was preferable to accept the view that the substance was a true metabolite. The results with S-52 provide excellent evidence for the correctness of this interpretation. The amount of dehydroisoandrosterone produced by this patient during the first control period was about 35 mg. per day and the 7-keto-

probably produced by the adrenal rather than as a secondary metabolite of peripheral tissues. Among the carcinoma patients of this series, only Subjects S-52 and L-7 exhibited elevated amounts of this metabolite. During the first course of amphenone the excretion of 7 = OD by Subject S-52 remained essentially at the control level, whereas the excretion of dehydroisoandrosterone was diminished to less than 10 mg. per day; in Subject L-7, similarly, there was little change in the production of 7 = OD during administration of amphenone despite a transient effect of the drug on dehydroisoandrosterone. When Subject S-52 received a second course of amphenone, the tumor and its metastases had materially altered secretory capacity, as shown in Figures 1 and 2. At this time amphenone caused a decrease in 7 = OD excretion to about half the control level. By contrast, however, dehydroisoandrosterone was less than one-tenth of the control value.

Androsterone (A)

The production of this metabolite was characterized by wide individual variations among the patients in this series. Subject G-37 exhibited essentially normal production whereas Subject D-41 showed the highest level—approximately 30 mg. per day. Since, however, Subject L-7 was a child, it is clear that in relation to her age the amount of A was the greatest in any of the patients studied. *In all the patients, amphenone lowered the formation of A, although not to the same extent that was evident with the 11-oxygenated metabolites.*

Etiocholanolone (E)

In all of the patients in this series there was an increase in the formation of this metabolite when compared with normal women of comparable age. However, as with A, there were wide individual differences. Thus, in Subject G-37 the production of E was 5.4 mg. per day during the control period —a normal value for girls in the early twenties. Subject D-41, with approximately 100 mg. per day, represented the other extreme in this series.

The effect of amphenone on the production of E was markedly different among this group of patients. Subject G-37, who received the smallest dose,

dehydroisoandrosterone amounted to almost a third of this value; during the second treatment with amphenone, dehydroisoandrosterone was about 7 mg. per day but 7-ketodehydroisandrosterone was nearly 6 mg. If the α,β unsaturated ketone had been formed as an artifact, it would be anticipated that the relative proportions of the 2 steroids would have remained virtually similar during both periods, since the manipulation of the extracts was identical insofar as possible. As indicated previously, this was not the fact. In other patients in whom dehydroisoandrosterone production was at an even greater level than in Subject S-52 (*e.g.*, Subject D-41) there was no concomitant production of significant amounts of 7-ketodehydroisoandrosterone. This is further verification of the conclusion that 7-ketodehydroisoandrosterone is a true metabolite rather than an artifact.

showed only a minor fall in the production of this metabolite. In Subject L-7 the excretion of E actually increased from 28.5 mg. per day during the control period to 42 mg. per day during the last period of amphenone administration. In Subject S-52, during the first course of amphenone treatment, the amount of E was intermediate between the levels in the 2 control periods (preceding and following administration of the drug). In these patients it must be concluded either that amphenone failed to suppress the biosynthesis of the precursor of this metabolite or that there was an increase in production despite the suppression of other adrenal hormones. In the other 2 patients there was a marked diminution in the production of E during administration of amphenone. The results in Subject P-51 are especially interesting, in that the progressive decline in E as well as of the other metabolites with continued administration of amphenone, is clear evidence of cumulative action. This is significant in view of the relatively rapid elimination of the drug and its metabolites. The prompt return to pre-treatment levels after cessation of treatment in 2 subjects (P-51 and the previously reported patient, R-42 (3, 4)) in whom E was greatly suppressed, is also clear evidence that amphenone altered steroid biosynthesis and was not lethal to the neoplastic cells.

The carcinoma of Subject S-52 exhibited 2 dissimilar responses at different times to the same agent. This is a highly significant result because of the implications that can be derived from the response of the metastases in different stages of development and function. During the first course of amphenone in Subject S-52, there was an increase in the excretion of E. At a later time when the disease had progressed materially, as judged not only from the quantitative changes in steroid production but also from the clinical condition, amphenone caused a distinct reduction in the amount of E. The decrease was from more than 30 mg. per day to less than 10 mg. per day (Fig. 2). This is the only patient in whom courses of amphenone were studied during different phases of the disease. For this reason generalizations were unwarranted, but it is clearly established that the carcinoma showed a grossly different behavior at 2 stages of its progress toward termination of the patient's life. It seems highly probable that other similar neoplasms may show altered responsiveness to both hormones and drugs at various stages of the disease. In this respect Subject S-52 may be considered comparable to previously reported Subject R-42 (3, 4) who exhibited gross alteration in the pattern of steroid production when the tumor was in the "localized phase" as compared with the later pattern when the cancer was widely metastatic.

Thus it is evident that amphenone decreased the production of etiocholanolone in the majority of patients, although in 2 subjects there was an absolute increase. In 1 of these 2 patients, at a later stage of the disease, there was a marked diminution in E during administration of amphenone.

COMMENT

By separation of the individual components of the urinary "17-ketosteroids" in a series of 5 patients with widespread metastatic adrenocortical carcinoma, it has been shown that *in all instances some or all of the components were decreased during treatment with amphenone.* In most patients reported, the diminished production and output extended to both chemical classes of steroid metabolites, *i.e.*, compounds oxygenated at C-11 of the steroid nucleus, as well as the 11-deoxyketosteroids. The significance of the results lies in the demonstration that amphenone invariably lowers the adrenocortical production of 11-oxygenated adrenal hormones. In addition there is often, though not invariably, a pronounced diminution in the 11-deoxysteroids. The distinction between these effects of the drug can be recognized only by separation of the individual steroids which comprise the "17-ketosteroids." This valuable information is obscured or lost completely by the application of the group reaction employed in the routine clinical determination of this mixture of compounds. Together with other evidence reported elsewhere (12, 13), these results support the conclusion that the biosynthesis of adrenocortical hormones results in 2 different classes of steroids, each subject to different control mechanisms. These control mechanisms can be differentially altered, even in a neoplastic tissue.

The interest in amphenone is not primarily because of any value in the treatment of carcinoma. Its importance stems from the fact that this relatively toxic compound, which has a number of undesirable side-effects, can materially alter one or both pathways in the biosynthesis of adrenocortical hormones leading to hydrocortisone and related 11-oxygenated hormones on the one hand, and 11-deoxysteroids or "adrenal androgens" on the other. Rosenfeld and Bascom (14) in studies of the isolated perfused calf adrenal concluded that amphenone exerts a relatively selective inhibition of steroid biogenesis, somewhat similar to the alterations in steroid metabolism in human subjects, as described in the present report. With a better drug, it should be possible to achieve these or related effects without the disagreeable reactions which limit application of the present compound. A means for the control of either component of the adrenal steroid secretion through such an agent should have the most profound and far-reaching implications for biology and medicine. By application of the refined chemical techniques available today, the proper evaluation of such compounds can be readily achieved. It is important that their real potential be recognized. Valuable information can be lost by the use of simpler but less reliable group reactions, which may obscure rather than clarify the underlying phenomena.

Acknowledgment

I wish to express my deep appreciation to the clinicians who by their cooperation made this study possible. At this Institute these were Drs. Leon Hellman, Olof Pearson, C. D. West and Mortimer Lipsett; at the Presbyterian Hospital, Dr. Sidney Werner; and at the Peter Bent Brigham Hospital, Dr. George Thorn with the assistance of Drs. Attallah Kappas, W. J. Reddy, Edna Sobel and Albert Renold. In the chemical work I wish to thank the technicians who carried out the analyses, especially Ruth Jandorek, Mildred Smulowitz and Voldemar Bankers.

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COMPARISON OF THE METABOLIC EFFECTS OF DIFFERENT THYROID PREPARATIONS*

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ABSTRACT

Rapid onset of increased nitrogen excretion following the daily administration of desiccated thyroid and diminution of the catabolic effect immediately upon cessation of therapy appeared inconsistent with the concept that this thyroid preparation is slow to act and prolonged in its effect. Consequently the effects of single, large, physiologically-comparable doses of triiodothyronine, thyroxine and desiccated thyroid were studied in 6 myxedematous and in 2 euthyroid subjects. All 3 preparations were similar with regard to rapidity and duration of action, as judged by such indices of metabolic acceleration as nitrogen excretion, BMR, serum cholesterol level and pulse rate. In the patients with myxedema, identical clinical responses followed treatment with triiodothyronine and thyroid. The differences in response, as determined by the various indices of metabolic acceleration, were more pronounced between the subjects than between the compounds. Triiodothyronine induced a minimal rise in the serum PBI level, whereas desiccated thyroid induced a rise which was more consistent with other indices of metabolic acceleration. It is suggested that the calorigenic activity of desiccated thyroid is dependent on its content of, or rapid conversion to, triiodothyronine and thyroxine.

Recent emphasis on the potency of triiodothyronine has engendered the belief that its effects are strikingly different than those of desiccated thyroid and has encouraged the notion that it is a more effective therapeutic agent. The similarities between various thyroid compounds deserve equal stress.

THE general belief that desiccated thyroid hormone is slow to exert metabolic acceleration, and yet prolonged in its duration of action (1, 2), has been strengthened by observations concerning the rapid activation of metabolic processes and equally speedy cessation of stimulation induced by triiodothyronine (3). As judged by nitrogen balance, increased oxygen consumption and certain clinical observations, thyroxine has ap-

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peared intermediate in its effects (4, 5). Older studies, utilizing measurement of the metabolic rate, indicated a duration of action comparable to that of desiccated thyroid (6, 7) whereas more recent studies have suggested that the actions of thyroxine more closely simulate those of triiodothyronine (8, 9).

Although most studies of triiodothyronine have indicated close similarity between its action and that of thyroxine, the current literature implies a much different action than that of desiccated thyroid. This opinion has been based primarily on clinical appraisal and measurement of the metabolic rate (10, 11). Recent and enthusiastic clinical application of triiodothyronine with the implication that its action is strikingly different than that of desiccated thyroid indicates the pertinency of emphasizing the similarity of the pattern of metabolic acceleration following single large doses of these 2 compounds.

MATERIALS AND METHODS

Six patients with primary thyroid failure and 2 obese euthyroid subjects were studied while on the metabolic ward and maintained at a relatively constant level of activity. Three of the myxedematous patients had received no therapy, and treatment had been abandoned in the others for a sufficient interval to permit development of the typical laboratory and clinical pattern of myxedema. Balanced liquid formula diets were used in all studies, caloric and protein content being calculated from standard reference sources (12–14). Periodically throughout, half-portions of the daily diet were analyzed for nitrogen. Measurement of nitrogen was made on 24-hour or 48-hour urine collections and, in part of the studies, on stools collected for periods of four to six days. When no stool analyses were made the fecal excretion of nitrogen was assumed to average 1.3 Gm. per day. In all studies, correction was made for nitrogen lost through blood sampling. Body weight was obtained each morning before feeding and after voiding.

Pulse rate was measured every two to six hours as the mean of two 60-second counts and was charted as the mean of all determinations for the specific day of study. The concentration of serum protein-bound iodine (PBI) was measured by the method of Barker (15), and of serum cholesterol by the method of Bloor (16). The basal metabolic rate (BMR) was obtained with a clinical closed-circuit apparatus, each value representing the average of at least 2 four-minute periods. Nitrogen in the diet, stools and urine was measured by the micro-Kjeldahl method.

Thyroxine (T_4) was administered orally as the sodium salt of *l*-thyroxine; *l*-triiodothyronine (T_3) was injected subcutaneously as a well-mixed suspension in sterile saline. The desiccated thyroid (thyroid) was a standard commercial product.[1]

RESULTS

The changes shown in Figure 1 are representative of those reported to accompany the use of large doses of T_3 and comparatively smaller amounts of thyroid. This 50-year-old woman (Case 1) with primary thyroid failure

[1] We are grateful to the Travenol Laboratories, Smith, Kline and French Laboratories and the Armour Laboratories, respectively, for supplying these preparations.

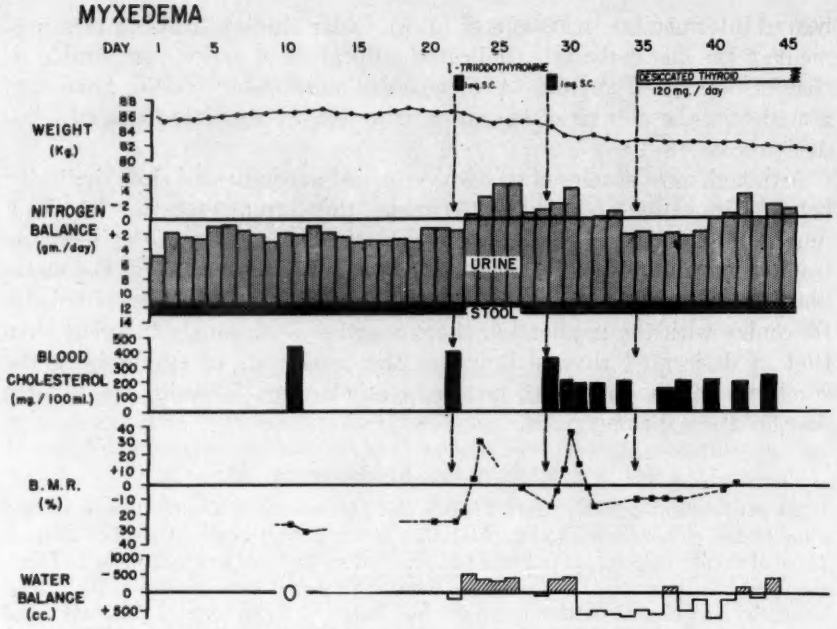

FIG. 1. (*Case 1*) Response to single large doses of triiodothyronine and small amounts of desiccated thyroid in myxedema.

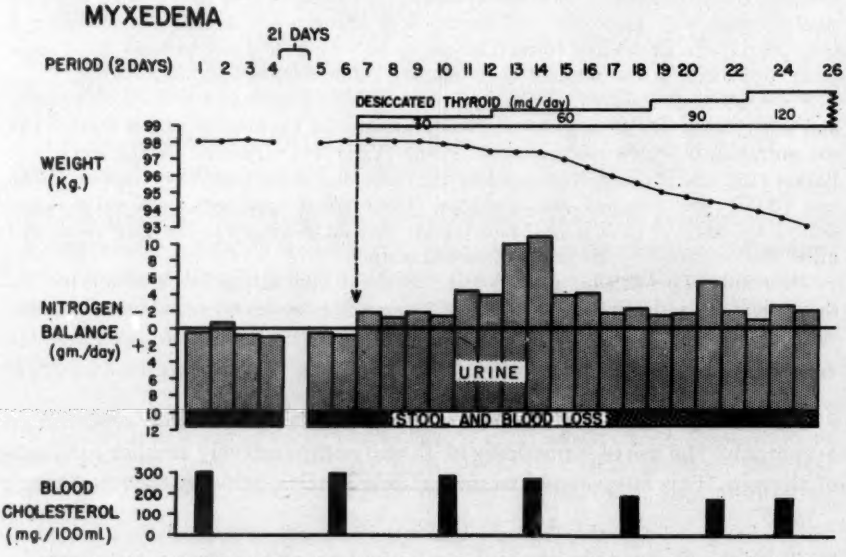

FIG. 2. (*Case 2*) Effect of desiccated thyroid on nitrogen balance in myxedema.

demonstrated the positive nitrogen balance so frequently noted in myxedema (17). Two subcutaneous injections of 1.0 mg. of T_3 resulted on each occasion in a rise in nitrogen excretion lasting five or six days. The BMR rose and fell rapidly, although not decreasing to pretreatment levels on either occasion. Metabolic acceleration, as judged by the increase in nitrogen excretion and rise in BMR, occurred much more slowly when maintenance doses of thyroid were given. Changes in water balance were closely correlated with the increased nitrogen excretion.

Figures 2, 3, and 4 show the effect of continuous administration of thyroid on nitrogen balance in 1 myxedematous and in 2 euthyroid subjects. The patient represented in Figure 2 (Case 2) was a 44-year-old man with post-thyroidectomy myxedema of several years' duration. He had received no medication for eight weeks prior to this study. Nitrogen excretion increased within two days after institution of therapy, reached a peak on the fourteenth day and then slowly decreased in intensity despite an increase in the dosage of thyroid. In the 2 euthyroid subjects represented in Figures 3 and 4 (Cases 3 and 4) there was a similar catabolic effect and maximal nitrogen excretion occurred at about the same time as was noted in the case of myxedema. The degree of enhanced nitrogen excretion was less in the 2 euthyroid subjects than that noted in the myxedematous patient, even though the latter received much smaller doses of hormone. As shown in both Figure 3 and Figure 4, there was a prompt decrease in nitrogen excretion when treatment was terminated.

Figure 5 illustrates the effects of a single large dose of thyroid, considered to be physiologically equivalent to 1 mg. of T_3, in a patient with myxedema. This 62-year-old woman (Case 5) had the typical features of primary thyroid failure of approximately twenty years' duration, and had received no thyroid medication for one year prior to study. Following the administration of a single dose of 1,500 mg. of thyroid by mouth there was an immediate increase in nitrogen excretion. The catabolic response lasted ten to eleven days and reached a peak on about the sixth day following treatment. Although the increase in serum PBI concentration closely paralleled the increase in nitrogen excretion, the basal metabolic rate attained its maximum more quickly, as was true after administration of T_3. An increase in pulse rate, noted on the day thyroid was given, reached a maximum on the following day; tachycardia persisted for only five days, showing a less prolonged effect than the other indices of metabolic acceleration. A rise in body temperature occurred on the day following treatment and was maintained for only three days; no significant febrile response was noted in the other studies. In view of the fact that this dose of thyroid induced metabolic acceleration of a degree and duration comparable to that reported after the administration of a physiologically comparable amount

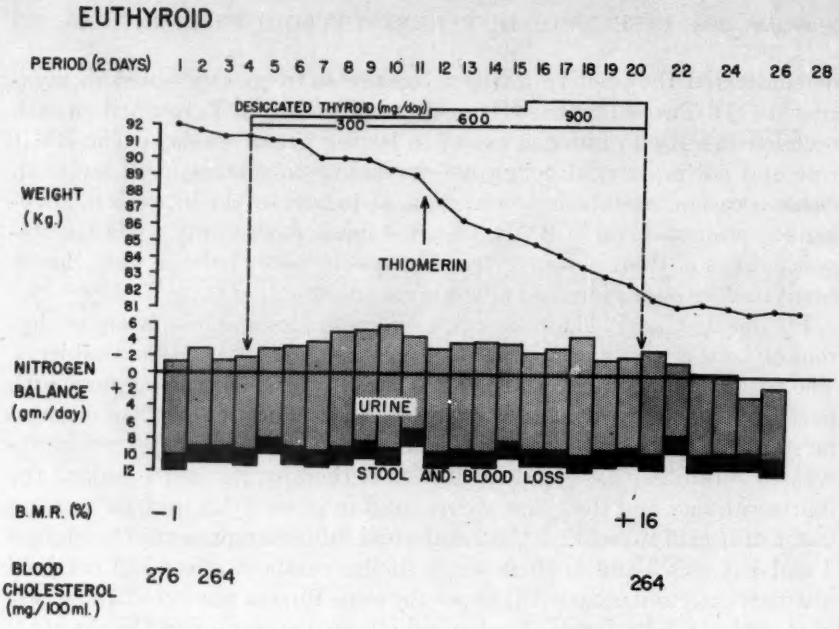

FIG. 3

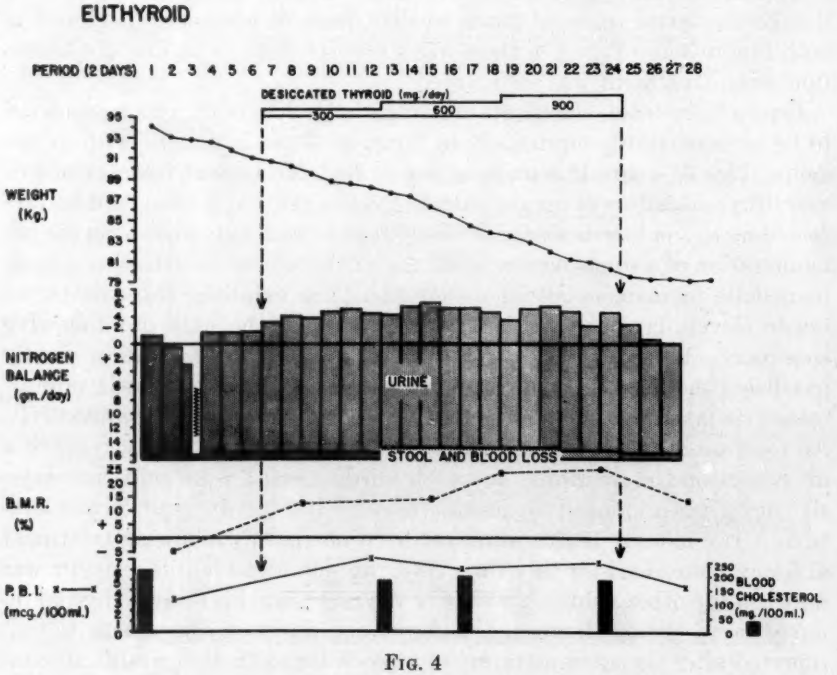
EUTHYROID
PERIOD (2 DAYS) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
DESICCATED THYROID (mg./day)
300
600
900
WEIGHT (Kg.)
NITROGEN BALANCE (gm./day)
URINE
STOOL AND BLOOD LOSS
B.M.R. (%)
P.B.I. (mcg./100ml.)
BLOOD CHOLESTEROL (mg./100ml.)

FIG. 4

FIGS. 3 and 4. (*Cases 3 and 4*) Effect of desiccated thyroid on nitrogen balance in euthyroidism.

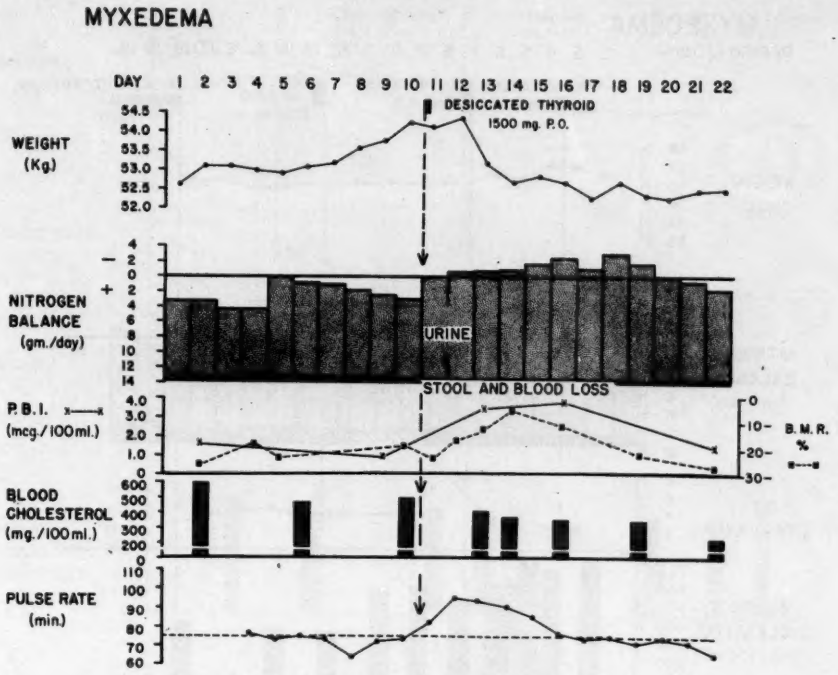

FIG. 5. (*Case 5*) Metabolic response to a single large dose of desiccated thyroid in myxedema.

of T_3, it appeared desirable to compare the effects of single doses of thyroid, T_3 and T_4 in the same subjects.

Figure 6 illustrates the metabolic effects of these 3 different thyroid preparations in a 62-year-old woman with thyroid failure secondary to chronic thyroiditis and apparently unrelated idiopathic hypercholesterolemia (Case 6). The strikingly positive nitrogen balance lessened during the first two days after the subcutaneous injection of 1 mg. of T_3, following which there was negative nitrogen balance. The seven-day interval between the administrations of T_3 and T_4 was insufficient to permit evaluation of the total duration of the catabolic effect following treatment with T_3; however, it did not attain a maximum for at least six days. A 2-mg. oral dose of T_4 appeared to have an immediate effect which lasted approximately ten days. A 1,200-mg. dose of thyroid caused an immediate metabolic acceleration, maximal in six days and lasting ten to eleven days. Although the degree of increase in metabolic rate was more marked following administration of T_3, the total pattern of response was similar with each thyroactive preparation. The marked rise in the level of serum PBI with

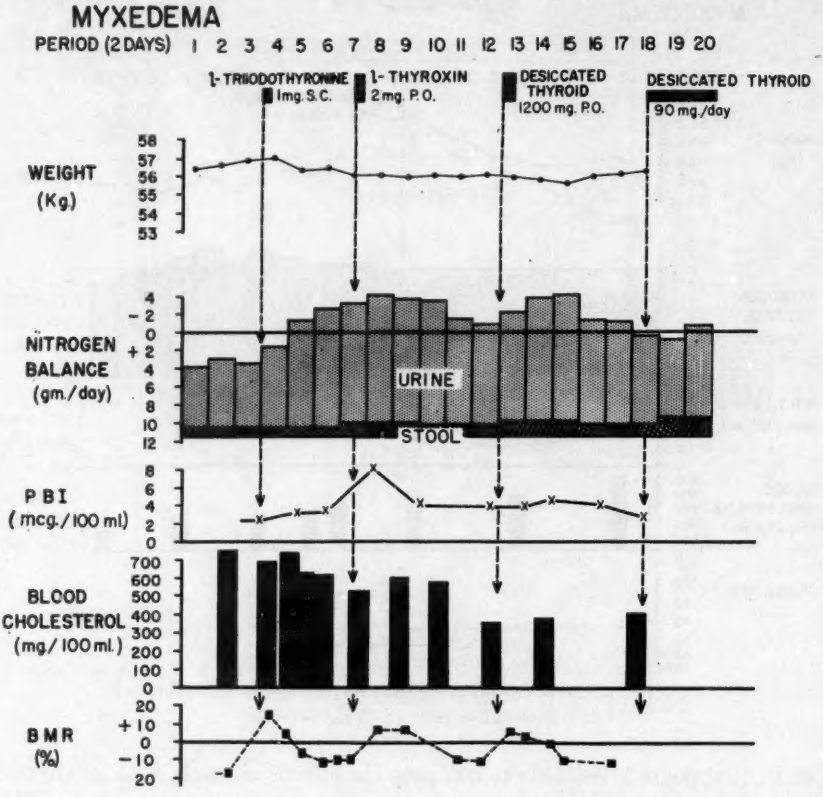
MYXEDEMA
PERIOD (2 DAYS) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
l-TRIIODOTHYRONINE 1mg. S.C.
l-THYROXIN 2mg. P.O.
DESICCATED THYROID 1200 mg. P.O.
DESICCATED THYROID 90 mg./day
WEIGHT (Kg)
58 57 56 55 54 53
NITROGEN BALANCE (gm./day)
4 -2 0 +2 4 6 8 10 12
URINE
STOOL
PBI (mcg./100 ml)
8 6 4 2 0
BLOOD CHOLESTEROL (mg./100 ml.)
700 600 500 400 300 200 100 0
BMR (%)
20 +10 0 -10 20

FIG. 6. (*Case 6*) Comparison of the effects of single doses of different thyroid preparations in myxedema.

T_4 and the lack of such response with T_3 are in accord with the results of other investigators. The minimal rise of the PBI level after administration of thyroid suggests a preponderance of T_3 in the whole gland. However, in other subjects, thyroid caused a more substantial increase in the PBI level (Figs. 5 and 9).

Figure 7 shows the comparative effects of T_3, T_4 and thyroid on weight loss, pulse rate and BMR in an obese euthyroid woman (Case 3). Cardiac acceleration was similar with all 3 compounds as regards duration of effect. The speed of action appeared to be the same with T_3 and T_4, but slightly slower with thyroid, although the greatest increase in pulse rate occurred with thyroid. Similarly the BMR rose at the same rate and to the same degree with both T_3 and T_4, although it remained at a higher level after T_4. With both types of thyroid hormone, elevation of the BMR was maintained for the six days measurement was continued.

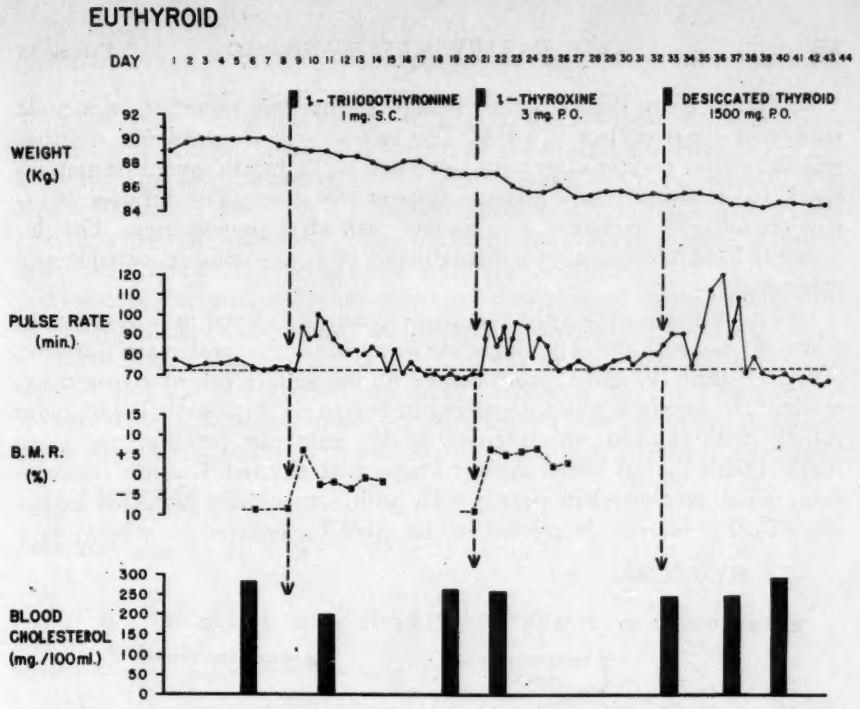

FIG. 7

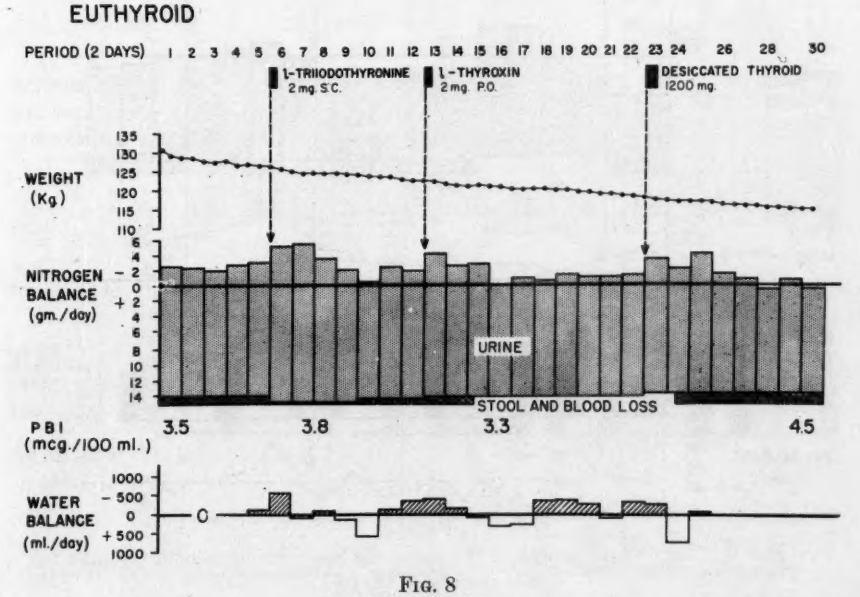

FIG. 8

FIGS. 7 and 8. (*Cases 3 and 4*) Comparison of the effects of single doses of different thyroid preparations in euthyroidism.

In Figure 8 are depicted the changes in nitrogen excretion in a male euthyroid obese subject (Case 4). The intensity of the catabolic reaction was somewhat greater after administration of T_3, but it should be emphasized that in this instance a larger amount was given. The duration of effect on nitrogen excretion was similar with all 3 preparations. The decrease in fecal nitrogen after administration of T_3 was not duplicated in any other study.

In Figure 9 are shown the metabolic changes induced in a 32-year-old white woman with primary idiopathic myxedema of several years' duration (Case 7). Both T_3 and thyroid caused an immediate rise in nitrogen excretion, the maximal effect occurring in five to six days with T_3 but more rapidly with thyroid; the intensity of the catabolic reaction was more marked with T_3, but lasted slightly longer with thyroid. Cardiac acceleration, which was equal in degree with both compounds, persisted longer after T_3. The increase in metabolic rate after T_3 persisted for several days

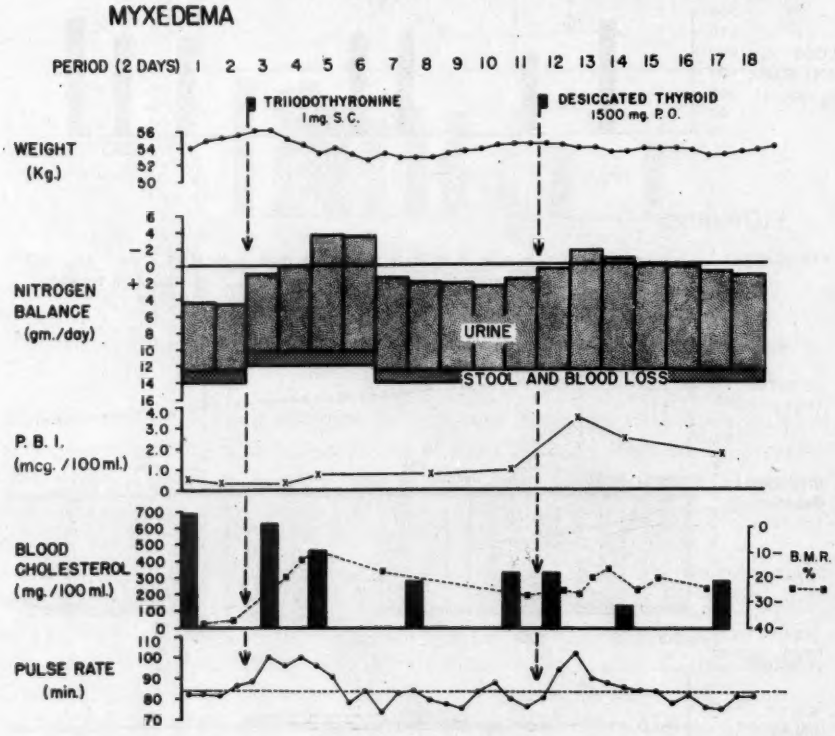
MYXEDEMA
PERIOD (2 DAYS) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
TRIIODOTHYRONINE 1 mg. S.C.
DESICCATED THYROID 1500 mg. P.O.
WEIGHT (Kg.)
56 54 52 50
NITROGEN BALANCE (gm./day)
6 4 −2 0 +2 4 6 8 10 12 14 16
URINE
STOOL AND BLOOD LOSS
P. B. I. (mcg. / 100 ml.)
4.0 3.0 2.0 1.0 0
BLOOD CHOLESTEROL (mg. / 100 ml.)
700 600 500 400 300 200 100 0
B.M.R. %
0 10− 20− 30− 40−
PULSE RATE (min.)
110 100 90 80 70

FIG. 9. (*Case 7*) Comparison of the metabolic responses of triiodothyronine and desiccated thyroid.

after the protein catabolic effect had waned. The response of the BMR to thyroid was less marked, but it should be noted that the original control level had not been regained at the time thyroid was administered. The serum PBI level showed little change following administration of T_3 and rose into the euthyroid range after the administration of thyroid, paralleling closely the rise and fall in urinary nitrogen excretion.

Assuming that a myxedematous subject should respond more vigorously to the initial calorigenic agent, particularly as concerns nitrogen excretion, a similar study was conducted in which the order of administration of thyroid and T_3 was reversed, as shown in Figure 10. This 48-year-old woman (Case 8) had postoperative myxedema of eleven years' duration. Although she had received no treatment for ten months, she was the only

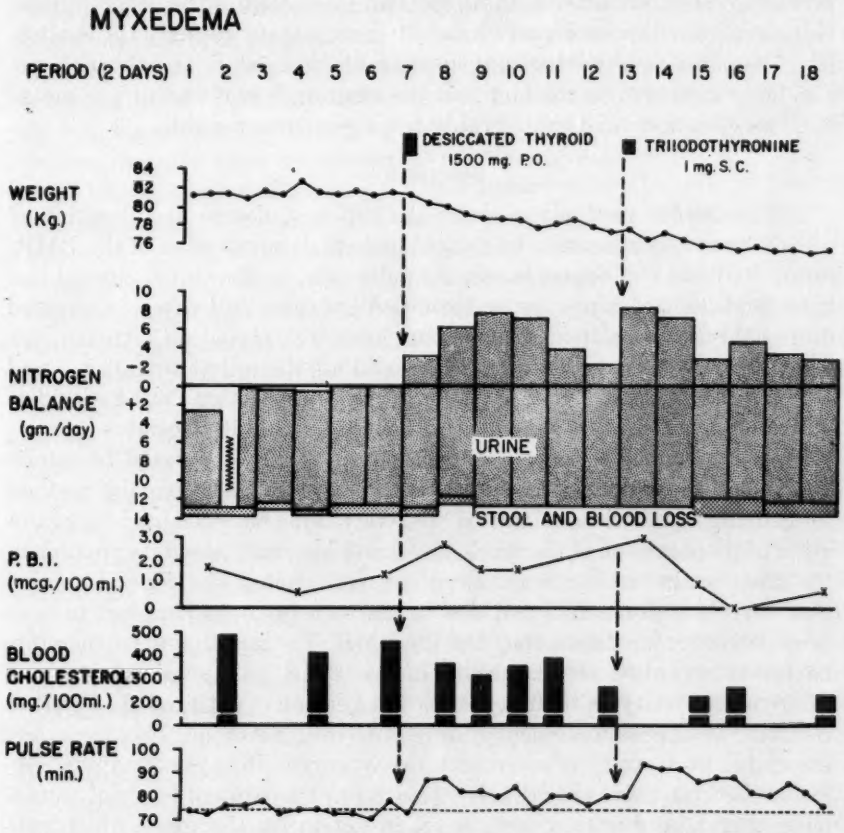

FIG. 10. (*Case 8*) Comparison of the metabolic responses to desiccated thyroid and triiodothyronine.

patient with thyroid deficiency in this study in whom nitrogen balance was not positive. The spacing between administration of the 2 preparations was planned to permit re-equilibration of nitrogen balance, but such was not attained after treatment with either thyroid or T_3; consequently, the duration of the catabolic effect could not be determined accurately but was in excess of twelve days with each compound. Nitrogen excretion rose immediately in each instance, becoming maximal within two days after T_3 but not until five or six days after thyroid. Despite the more rapid response to T_3, it is interesting that nitrogen loss was more marked after thyroid (which was administered first) than after T_3, whereas in the previous study (Fig. 9), nitrogen loss was greater after T_3 when this preparation was used initially. Figure 10 also shows that in Case 8 there was a longer period of cardiac acceleration after both drugs than was noted in the other studies. In general, the data of Figures 9 and 10 demonstrate not only the similarity of response to physiologically comparable doses of T_3 and thyroid, but also focus attention on the fact that the magnitude and duration of metabolic acceleration vary considerably from person to person.

DISCUSSION

The numerous methods whereby the rapidity, degree and duration of metabolic acceleration may be gauged include determination of the BMR, serum PBI and cholesterol levels, the pulse rate, various other clinical features, and changes in nitrogen excretion. All of these indices were measured during this study, although major emphasis was placed on nitrogen balance. There was considerable variation in all these determinations, and moreover the response to all types of thyroid preparation varied considerably among individuals, as noted by Blackburn and his associates (5).

Measurement of change in oxygen consumption, converted to caloric equivalent and expressed as the BMR, has been the most popular method for gauging metabolic acceleration. This technique has been used for evaluation of preparations of the whole gland and has maintained its popularity for investigation of the newer thyroid preparations. The general opinion that thyroid is slow to act and that its action is prolonged appears to have been derived from measurement of the BMR. The question must therefore be raised regarding the reliability of the BMR as a sensitive index of calorigenic activity. Although Means and Lerman (6) utilized the BMR to establish an approximate decay curve of thyroid hormone, Thompson and associates in a study of thyroxine decay curves in myxedematous subjects raised the point that the slow return to pretreatment levels of metabolism might be due to a process set in action by thyroxine which continued after the activity of the hormone had waned (18). Rabinowitch pointed out that the change in metabolism immediately following the ad-

ministration of thyroxine is so complicated by secondary or side-reactions that one cannot assume a direct relationship between heat production and concentration of thyroid hormone in body tissues (19). In our study, desiccated thyroid affected the BMR within two days, having a maximal effect in two to four days and a duration of action of from six to over ten days. T_3 acted within one or two days, had a maximal effect in two or three days, and maintained stimulation for from four to over nine days. The effects of T_4 were virtually the same as those of T_3. The intensity of the BMR response was more marked with T_3 but, as shown by Rawson *et al.*, the total change was no greater than those which followed the use of other thyroid preparations (4). Although effort was made to administer physiologically comparable amounts of thyroid hormone, this objective was probably not attained in all studies; relatively larger amounts of T_3 were given in most instances. Furthermore, the parenteral administration of T_3, as contrasted to the oral use of desiccated thyroid, would tend to amplify more rapid action of the former preparation. In short, although desiccated thyroid was somewhat slow to act and slightly more prolonged in its effects, there was no striking difference in the response of the BMR between the different preparations. In some instances the responses were identical.

As has been commented on by Asper and his associates, the increase in the concentration of serum PBI served as a poor index of calorigenic response (2, 3, 20). When triiodothyronine was administered there was little or no increase in the circulating hormone, whereas treatment with thyroxine resulted in a rise of the PBI level out of proportion to the degree of metabolic acceleration as judged by clinical response and other laboratory measurements. As noted by Winkler, Riggs and Man (21), a single large dose of desiccated thyroid caused an increase in the PBI level which fitted better with the total pattern of response. There appeared to be little difference between thyroid, T_3 and T_4 concerning the rapidity or degree with which the hypercholesterolemia of myxedema was reduced to, or toward, normal levels.

Cardiac acceleration appears to be a sensitive and reproducible index of metabolic stimulation and the striking similarity in response in pulse rate to the different thyroid preparations was much more impressive than any difference in response. Although in some patients there appeared to be a more rapid development of tachycardia after T_3, in others it occurred more quickly after thyroid. The time of maximal effect was comparable with all 3 preparations and the duration of the tachycardia was similar with T_3 and with thyroid.

Changes in mental and physical activity and in the color, warmth and moisture of the skin were followed in all subjects. In the myxedematous patients evaluation was also made of the effects of the 3 preparations on the

quality of the voice, and changes in the paresthesias and the pseudomyotonic relaxation phase of the deep tendon reflexes. The responses to each hormone were compared with regard to time of onset, day of maximal change, intensity of effect and duration of action. The intensity of effect was more marked in the myxedematous patients, but there was no clear-cut difference between the 3 types of thyroid hormone. With each compound in all subjects, objective changes were noted within fourteen to twenty-four hours, and the maximal effect was attained between the third and fifth day after therapy. The duration of the changes ranged from four to nine days. An effect on the slowed relaxation phase of the deep tendon reflexes was not noted until four to five days had elapsed; it was maintained for nine to eleven days after the single dose of thyroid hormone. Severe headache occurred in 3 myxedematous patients, and vomiting in 1. No unpleasant symptoms were noted in the other subjects.

The major emphasis during this comparative study was placed on change in nitrogen balance. Not only was this technique one of the main indices of metabolic acceleration to be applied when thyroid hormone was first under investigation, but it ranks as a highly accurate method of measuring an increase in oxidative mechanisms. Under the conditions of careful balance study, nitrogen excretion serves as a precise measure of onset, degree and duration of the action of thyroid hormone. Although a direct effect of thyroid hormone on protein tissue has been suggested, particularly in myxedema (22), an equally plausible explanation for the catabolic reaction rests in the caloric discrepancy induced by enhanced heat production. Thus the measurement of nitrogen balance should serve as a more accurate index than the measurement of oxygen consumption, or for that matter of any readily applicable indirect method of determining the increase in heat production. When desiccated thyroid was given to euthyroid or myxedematous subjects in relatively small and continued doses, there was an immediate effect. Even more interesting, and to us rather surprising, was the rapid termination of the catabolic reaction in the euthyroid subjects when treatment was discontinued. The time of maximal effect was similar in all subjects. As would be expected, whether one accepts the theory of the presence of a large amount of "deposit protein" (22, 23) or greater sensitivity to thyroid hormone (24), the intensity of the catabolic response was more striking in myxedema.

The changes in nitrogen balance when each of the 3 types of thyroid hormone was given in a single large dose were of sufficient degree to permit comparison of the effects. All 3 compounds elicited some response within one or two days, closer separation being impossible in most studies because of the length of the urine collection periods (forty-eight hours). In 1 of the 2 studies in which the urine collection period was twenty-four hours, T_3

had an effect on the first day; in the other study, nitrogen excretion did not increase until the second day. The maximal catabolic effect tended to occur within a shorter time after the use of T_3 or T_4 (average 4.0 days) than with desiccated thyroid (average 5.5 days). However, in comparing the individual responses, the range was about the same. The small number of studies prohibits statistical comparison.

The duration of the increased nitrogen excretion after administration of a single dose of these preparations was approximately ten to eleven days with thyroid and eight to nine days with T_3 and T_4. In no instance did the catabolic effects of thyroid terminate in less than nine days, whereas in several instances the increased nitrogen excretion induced by T_3 was of shorter duration. However, in some studies, the duration of the catabolic response to T_3 equalled that to thyroid. As was noted with the continuous administration of thyroid, the intensity of the catabolic response was greater in myxedema than in the euthyroid subjects, but the magnitude of the nitrogen excretion differed little between the 3 thyroid preparations. In general there was no striking difference in the catabolic responses to single large doses of thyroid, T_3 or T_4. Although the onset of action and duration of effect on nitrogen excretion were slightly shorter with triiodothyronine, even these changes were not constant.

The acute nature of the response to single large doses of thyroid indicates a latent period and duration of activity similar to those noted with triiodothyronine or thyroxine. One might suggest that the thyroid contained, in addition to a long-acting component, sufficient T_3 or T_4 to promote the immediate and vigorous increase in nitrogen excretion which followed its administration in a single dose. If this were true there should have been a persistent catabolic effect following the initial sharp response, due to conversion of the slower-acting components of desiccated thyroid to active hormone. This phenomenon was not observed. Furthermore one would not expect the rapid cessation of the catabolic effect which followed termination of continuous thyroid medication if there existed both rapid and slow-acting components in the whole-gland preparation. The type of metabolic acceleration produced by desiccated thyroid, coupled with the response of the serum PBI level to different thyroid preparations, is more suggestive that the active components of desiccated thyroid represent a combination of T_4 and T_3.

In view of the fact that the single doses used in this study were very large, and in this sense unphysiologic, it is possible that the sudden "toxic" stimulus induced a sharp adrenocortical stimulus, with consequent increased nitrogen excretion. The fact that triiodothyronine has been shown to increase nitrogen excretion in a hypoadrenal patient maintained on constant amounts of cortisone renders this possibility very unlikely (25).

Measurement of blood and urinary 17-hydroxysteroid levels in 2 of our patients before and after treatment with thyroid yielded no evidence of any increase in production or excretion of antianabolic steroid hormones.

The introduction of triiodothyronine has stimulated some revision of older ideas concerning the nature of the thyroid hormone. Particular stress has been laid upon its marked potency, and this fact appears to be indisputable. Considerable emphasis has been laid on its apparent rapid and short-lived activity, and some opinion has been offered that it might, possibly by rapid cellular penetration, be more effective than other thyroid preparations in some cases of myxedema and in many patients with a poorly defined state termed hypometabolism (26). Although several authors have demonstrated a similar latent period and a relatively similar duration of action between T_3 and T_4, no comparison has been made of the actions of T_3 and commercial desiccated thyroid in these respects. The assumption that there is a difference is based partly on clinical observation and partly on comparison of the actions of thyroid and thyroxine. The studies reported here, although attesting to the marked potency of triiodothyronine, do not indicate that it is strikingly different from desiccated thyroid, either in rapidity of action or duration of effect. The similarity of these preparations deserves more emphasis. Moreover, care should be taken to avoid invidious comparison of the different types of thyroid compounds unless the doses used are physiologically comparable, and the techniques of evaluation broad in their objectivity.

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THE MICRODETERMINATION OF TESTOSTERONE IN HUMAN SPERMATIC VEIN BLOOD*

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ABSTRACT

A procedure for microdetermination of testosterone in human spermatic vein blood is presented. C^{14}-testosterone was added to the plasma to facilitate chemical studies. The plasma was extracted with ether and partitioned between petroleum ether and 70 per cent ethanol. The alcohol fraction was treated with 2,4-dinitrophenylhydrazine and the derivative was chromatographed on 2 different columns to constant specific activity. The validity of the method was tested further by changing the derivative obtained from dog plasmas to the free steroid, re-processing it, and demonstrating that the specific activity was not changed by these procedures.

Data obtained from clinical material showed that the level of testosterone in human spermatic vein blood varies from 1.6 to 0.025 μg. per cc. of plasma, and decreases with aging.

TESTOSTERONE has been identified in large pooled samples of spermatic vein blood from the human subject by Lucas *et al.* (1) and from the dog by West *et al.* (2). An increase in the testosterone content of dog spermatic vein blood during treatment with chorionic gonadotropin has been demonstrated by the Hollanders (3) and by Brinck-Johnsen and Eik-Nes (4).

In order to study the testosterone level in human spermatic vein blood under various physiologic conditions as well as in carcinoma of the prostate, it was found necessary to develop a method for the analysis of amounts of plasma ranging from 2 to 5 cc. This report presents the procedure for the microdetermination of testosterone in plasma, evidence for its validity, and data from clinical material.

MATERIALS AND METHODS

Collection of blood

Spermatic vein blood was obtained at orchiectomy from patients with prostatic carcinoma and during inguinal hemiorrhaphy from control patients without any known

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endocrine disorder. Ten samples were obtained from different patients in each group. A sample of peripheral blood was also obtained at the time of surgery. Since the levels of testosterone in human and dog bloods were found to be of the same order of magnitude, pooled dog spermatic vein blood was used for the longer and more detailed procedure to demonstrate the validity of the shorter method used for the human studies. Spermatic vein blood was obtained from mature male mongrel dogs anesthetized with intravenous Nembutal. The spermatic veins were isolated and cannulated with a PE-190 polyethylene catheter. Blood was collected separately from 2 dogs. One dog was untreated; the other was given a single intravenous dose of 5,000 units of human chorionic gonadotropin[1] (HCG) before commencing the collection. Spermatic vein blood was collected continuously for two hours and totaled approximately 200 cc. for each animal.

Extraction

The human and dog bloods were treated identically after collection. An exact aliquot of plasma was measured and diluted with 4 volumes of physiologic sodium chloride. To this was added 2 μg. of C^{14}-testosterone,[2] which represented 23,000 counts per minute. The diluted plasma was extracted four times with 2 volumes of ethyl ether. The ether extract was washed with 0.1 *N* sodium hydroxide and water, and then evaporated to dryness under a stream of air. The residue was dissolved in 10 cc. of petroleum ether, allowed to stand overnight, and extracted three times with equal volumes of 70 per cent ethanol. The alcohol fractions were back-extracted with 10 cc. of petroleum ether. The combined ethanol fractions were evaporated to dryness. The sample was transferred to a 1-cc. glass-stoppered test tube by means of several washes of 95 per cent ethanol.

Estimation of ketonic steroids

The ketonic steroids were estimated by a modification of the procedure of Reich *et al.* (5). Each dry sample in the 1-cc. test tubes was dissolved in 50 μl. of 95 per cent ethanol.[3] To this was added 20 μl. of DNPH reagent[4] (prepared just before use in the proportions 4.0 mg. of dinitrophenylhydrazine (DNPH),[5] 1.0 cc. of 95 per cent ethanol, and 0.05 cc. of concentrated hydrochloric acid). The test tubes were stoppered and allowed to stand overnight at room temperature. The excess DNPH was then destroyed by adding 10 μl. of Benedict's solution[6] and heating in a boiling water bath for ten minutes. The reaction mixtures were diluted with 100 μl. of water and extracted by shaking with 0.3 cc. of toluene. A 250-μl. aliquot of toluene was removed into a glass-stoppered test tube and stored until the time of chromatography. Just before chromatography the samples were evaporated to dryness and dissolved in 0.2 cc. of 1:1 benezene-petroleum ether.

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[1] Human chorionic gonadotropin (APL) obtained through the courtesy of Ayerst, McKenna & Harrison.

[2] Testosterone-4-C^{14}, specific activity 10 μc./mg., obtained from Tracerlab, Inc., Boston, Mass. It was chromatographed on Whatman #1 paper in a toluene-propylene glycol system, eluted, and stored at 0° C. as an alcoholic solution.

[3] Calibrated Levy pipettes, obtained from A. H. Thomas Co., were used for measuring amounts ranging from 10 to 250 microliters.

[4] DNPH(2, 4,-dinitrophenylhydrazine).

[5] Eastman Kodak product recrystallized from *n*-butyl alcohol.

[6] Qualitative, made from reagent-grade chemicals.

This material was chromatographed on a column of 200 mg. of Fisher alumina prepared with 1:1 benzene-petroleum ether. The column was developed with A) 5 cc. of 1:1 benzene-petroleum ether; B) 6 cc. of 9:1 benzene-petroleum ether; and C) 10 cc. of chloroform. These fractions were evaporated to dryness and each was dissolved in 1 cc. of toluene. The optical density at 380 mμ was determined by means of a Beckman DU spectrophotometer. Radioactivity was measured by plating at infinite thinness and counting in a flow-gas counter.

Preliminary experiments with dinitrophenylhydrazones of known pure compounds showed that the androstenedione derivative was eluted in fraction B, and that fraction C contained a mixture of dinitrophenylhydrazones of testosterone and 7-ketocholesterol.

Second chromatography

Fraction C, which contained all the radioactivity, was rechromatographed on a column of 400 mg. of Woelm alumina containing 5 per cent water. Benzene was used as the eluting solvent and 1-cc. fractions were collected. When the first component was eluted the column was stripped with chloroform and ethanol. The fractions were read in a Beckman DU spectrophotometer at 380 mμ and an aliquot was taken for determination of radioactivity.

Figure 1 represents the second chromatography of the derivative from the blood of a HCG-stimulated dog. The coincidence in the first peak of radioactivity with optical density is typical of all the human samples studied.

The human bloods were processed up to this point. The amounts were found insufficient for further manipulation. However, with the more abundant dog bloods, other pro-

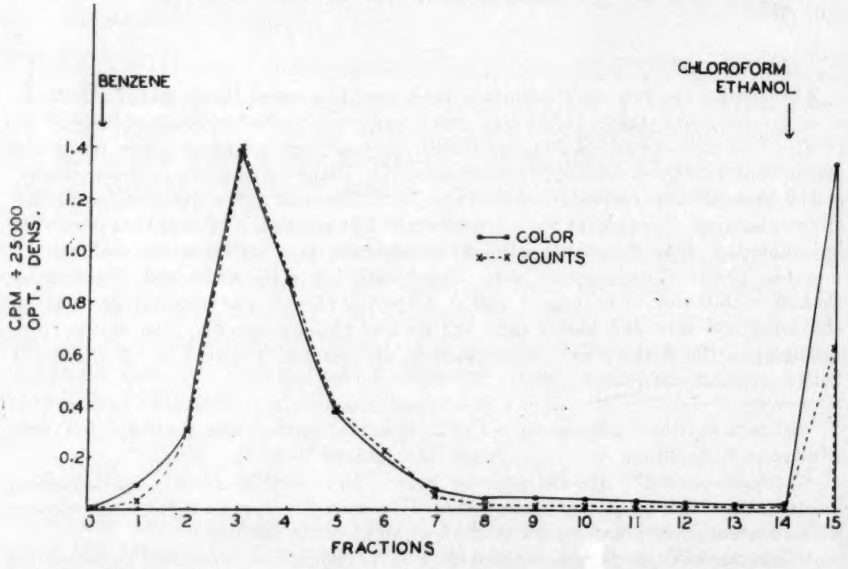

FIG. 1. Second chromatography of DNPH derivative from plasma of HCG-treated dog on a column of 400 mg. of Woelm alumina with 5 per cent water. The ordinate represents optical density and counts per minute divided by a factor which makes the peak radioactivity coincide with peak optical density.

TABLE 1. SPECIFIC ACTIVITY OF POOLED DOG SPERMATIC VEIN BLOOD (CPM/μG. TESTOSTERONE)*

Dog	After second chromatography as DNPH derivative	As free steroid after paper and column chromatography	Re-processed and chromatographed DNPH derivative
Stimulated with HCG	2400	2400	2700
Unstimulated	3300	3300	3100

* Specific activity of testosterone from spermatic vein plasmas of 2 dogs, estimated as DNPH derivative and as free steroid. The values represent total radioactivity divided by the total amount of testosterone (in micrograms), including C^{14}-testosterone.

cedures were carried out, to demonstrate that no further change in specific activity occurred.

The fractions from the second chromatography exhibiting constant specific activity were pooled, and the dinitrophenylhydrazones were decomposed to the free ketonic steroid with pyruvic acid by a modification of the method of Mattox and Kendall (6).

The free steroid was then chromatographed on Whatman #4 paper in a toluene-propylene glycol system. The strip containing radioactivity was eluted with 95 per cent ethanol and chromatographed on alumina as follows:

A slurry of 3 Gm. of Fisher alumina in petroleum ether was prepared. The column was developed with 10 cc. of petroleum ether, 20 cc. of 10 per cent chloroform in petroleum ether, 30 cc. of 25 per cent chloroform in petroleum ether, 70 cc. of 75 per cent chloroform in petroleum ether, and 50 cc. of chloroform.

Testosterone is eluted with 75 per cent chloroform in petroleum ether.

The specific activity of the testosterone was determined by estimating optical density at 240 mμ in a Beckman DU spectrophotometer, and the corresponding radioactivity.

The testosterone-band eluate was then re-processed with dinitrophenylhydrazine, chromatographed, and the specific activity re-determined.

Table 1 shows that the specific activity of testosterone obtained from dog spermatic vein plasma does not change significantly throughout 3 procedures. Therefore it seems reasonable to assume the purity of the testosterone derivative obtained after the second chromatography (first column) which was performed on the human bloods.

Calculations

The amount of testosterone in the spermatic vein plasma samples was calculated from the specific activity of the dinitrophenylhydrazone obtained after the second chromatography. Correction for recovery and for the added C^{14} testosterone was made, and the final figure was expressed as μg. of testosterone per cc. of plasma.

RESULTS

Spermatic vein blood from dogs was found to contain 0.2 μg. of testosterone per cc. of plasma. Stimulation with 5,000 units of HCG increased this value to 2.2 μg. per cc. Three additional experiments, using a dog as

it own control before stimulating with intravenous HCG, yielded similar results.

The values obtained on peripheral blood both from the human subject and the dog showed a specific activity identical with that of the added C^{14} testosterone, indicating that, by this method, testosterone cannot be demonstrated in peripheral blood.

Figure 2 shows the results obtained on 20 samples of human spermatic vein blood. In normal subjects, values ranged from 1.6 to 0.4 μg. per cc. and decreased with aging. Unfortunately no controls were available in the age group 70–80 years. Therefore comparison of the data with those for the prostatic carcinoma group could not be made. Two of the prostatic carcinoma patients had been treated with stilbestrol prior to orchiectomy—a 56-year-old man for one year and a 78-year-old man for one month. No correlation was observed between the concentration of testosterone and the histologic appearance of the testes.

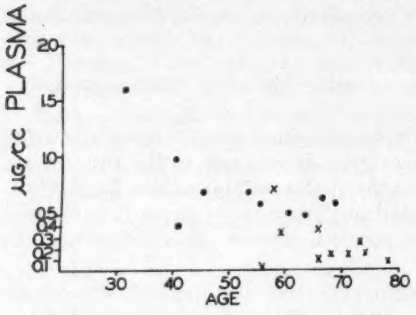

FIG. 2. Concentration of testosterone in human spermatic vein blood, expressed as μg. of testosterone per cc. of plasma, corrected for added C^{14} testosterone. ● = Normal controls. × = Patients with carcinoma of the prostate.

DISCUSSION

The method presented in this report has the advantage of measuring as little as 0.1 μg. of a specific steroid, and makes it possible to work with as small a volume as 2 cc. of plasma. The procedure appears to be valid for the determination of testosterone, on the basis of the constant specific activity of testosterone measured as the dinitrophenylhydrazone and as the free steroid.

The level of testosterone in human spermatic vein blood varies from 1.6 to 0.025 μg. per cc. of plasma. The decrease in values with aging is expected, in view of the known decrease in urinary 17-ketosteroids (7).

Acknowledgments

We wish to thank members of the Surgery and GU Departments for obtaining the human bloods; and Drs. Hanbury and Blanton for performing the surgical procedures on the dogs.

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METABOLISM OF PROGESTERONE-4-C^{14} IN A POSTMENOPAUSAL WOMAN WITH A BILIARY FISTULA*

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ABSTRACT

The distribution of radioactivity in bile and urine after injection of progesterone-4-C^{14} was studied as a function of time in a normal postmenopausal woman with a biliary fistula. The amounts of methylene chloride-soluble ("free") steroid and the material which became chloroform-soluble after hydrolysis with β-glucuronidase were measured and analyzed by paper chromatography. The major amount of radioactivity was excreted in the bile. Both bile and urine contained pregnanediol, pregnanolone and some unidentified polar material, all of which were released by β-glucuronidase hydrolysis. Characterization of pregnanediol and pregnanolone was based on chromatographic mobility and carrier experiments using authentic steroids.

INTRODUCTION

THE pioneer work of Venning and Browne (1) and of Dorfman, Ross and Shipley (2) identified pregnane-3α,20α-diol[1] and pregnan-3α-ol-20-one as metabolic products of progesterone in human urine, and subsequent work in many laboratories has confirmed these observations. However, it is generally agreed that something less than 20 per cent of the progesterone administered is recovered in the urine in the form of these 2 compounds (3, 4). The remaining 80 per cent must be eliminated either as other urinary metabolites or excreted by some other route. In more recent

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[1] Pregnane-3α,20α-diol = pregnanediol.
Pregnan-3α-ol-20-one = pregnanolone.

work, Gallagher and Bradlow *et al.* (5, 6) have shown that 37 per cent of administered radioactivity was excreted in the urine over a three-day period following the intravenous administration of progesterone-4-C^{14}. Davis *et al.* (7) and Pearlman (8) have reported excretion data following injection of radioprogesterone by the intramuscular route.

Both pregnanediol and pregnanolone have been found in hydrolysates of bile obtained from various species (9–11). Excretion studies in which labeled progesterone was given to rats showed that the biliary route was quantitatively more important than the urinary for this animal (12). Therefore, it seemed probable that biliary excretion would account for the major undetermined portion in human metabolic studies. Sandberg and Slaunwhite (13) have published results pertinent to this point and have furnished data on the excretion of C^{14}-progesterone metabolites in bile, feces and urine and on the rates of clearance of free and conjugated steroids from plasma. The present report provides quantitative information on the extent of biliary excretion in the human being and additional information on the nature of progesterone metabolites in bile.

MATERIALS AND METHODS

Purity of progesterone-4-C^{14}

Progesterone-4-C^{14} used in this study was synthesized (14), and had a specific radioactivity of 1.5×10^7 counts per minute per mg. On chromatographic analysis, the material exhibited a single peak of radioactivity having a mobility identical with that of authentic progesterone. In addition repeated recrystallizations of a mixture of authentic progesterone and the labeled hormone from different solvents failed to demonstrate any decrease in specific activity.

Clinical material and dosage

The subject used in these studies was a 60-year-old woman who had undergone cholecystectomy and insertion of a T-tube for drainage of bile. Prior to surgery a sulfobromophthalein retention test indicated normal liver function. The patient was given 0.36 mg. of progesterone-4-C^{14}, equivalent to 5.44×10^6 counts per minute, dissolved in 0.5 ml. of ethanol-saline solution. This was injected as a single dose into the antecubital vein. Bile and urine were collected during the first two 12-hour periods following administration. The 4 samples were stored at 1° C. until the time of analysis.

Radioactivity determinations

Aliquots of bile and urine were plated directly on aluminum discs and were counted in a windowless flow counter long enough to achieve a standard error in net activity of less than 5 per cent. The samples were corrected for self-absorption, and the count was expressed in terms of infinite thinness. The same method of estimating radioactivity was used throughout the entire study.

Identification of metabolites in urine

A 15-ml. portion of the first 12-hour urine sample was adjusted to pH 4.5 with acetate buffer (0.2*N*) and incubated with 75,000 Fishman units of beef liver β-glucuronidase

and a few drops of penicillin for eighteen hours at 47° C. Following incubation the solution was extracted with chloroform, and the extract was chromatographed in the Bush Skellysolve C:85% methanol system (15).

The remainder of the first 12-hour sample was extracted with methylene chloride for removal of free steroids, and the extract was chromatographed *in toto* in the Bush benzene-Skellysolve C:80% methanol (BC/80) system (15).

Identification of metabolites in bile

Following the C^{14}-assay, the first 12-hour sample of bile was lyophilized. The solid residue remaining was then triturated with organic solvents, beginning with methylene chloride and then methanol. After each extraction the solution was filtered free of the solid residue. At the conclusion of the methanol extraction, the remaining buff-colored solid material, which contained no detectable radioactivity, was discarded. Each organic fraction was then analyzed chromatographically, using the BC/80 system, before and after hydrolysis with β-glucuronidase. For hydrolysis, each extract was evaporated to dryness under nitrogen and dissolved in acetate buffer, pH 4.5; enzyme and penicillin were added, and the incubation and extraction proceeded as described for urine.

Identification of metabolites

Chromatography was carried out on strips of filter paper 2 or 3 cm. wide and 55 cm. long. At the conclusion of the run the papers were dried and the entire length of the strip was cut into 2-cm. sections. These sections were then counted in the windowless flow counter. Control strips containing authentic standards were run simultaneously with each unknown strip. Pregnanediol and pregnanolone[2] were located on the control strips with the phosphomolybdic-acid reduction test. Peaks of radioactivity having the same mobilities as pregnanediol and pregnanolone were eluted from the chromatogram sections with methanol. Carrier amounts of authentic steroid were dissolved in the radioactive solutions, and the specific activities of the crystalline mixtures were determined. The steroids were then recrystallized to constant specific activity from hexane or aqueous methanol.

As a final test for radio-purity of the material which had been crystallized to constant activity, the Gibbs' phase rule constant solubility test described by Gutmann and Wood (16) was used. In this test a solvent was selected in which the mixture of carrier and radioactive eluate was sparingly soluble; a convenient volume of the solvent was saturated with a small amount of the mixture by equilibration at constant temperature. A sample of the saturated solution was withdrawn and its radioactivity measured. A somewhat larger portion of the crystalline mixture was then added to the solution, and after a suitable interval for equilibration the radioactivity of the supernatant liquid was again determined. If the carrier were not identical with the radioactive eluate, addition of more of the mixture would increase the concentration of radioactivity in the liquid phase. If carrier and eluate were identical, the radioactivity of the 2 samples of solution would remain the same.

RESULTS

Excretion of radioactivity

The radioactivity measured in bile and urine during the 24-hour collection period is shown in Figure 1. The total radioactivity recovered was

[2] Kindly furnished by Dr. Max Tishler, Merck & Co. Inc., Rahway, N. J.

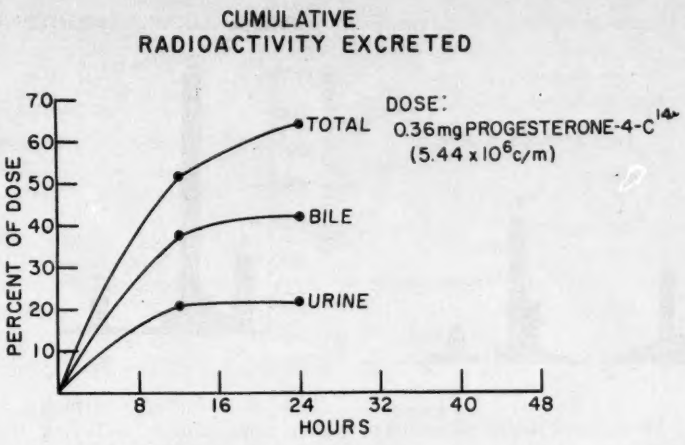

FIG. 1. The percentage of radioactivity excreted in the urine and bile following intravenous administration of progesterone-4-C^{14} in a normal postmenopausal woman with a biliary fistula.

3.50×10^6 counts per minute or 64 per cent of the administered dose. Of this total, the bile accounted for 65 per cent and the urine for 35 per cent.

Metabolites in urine

Methylene-chloride extraction of an aliquot of the first 12-hour sample of urine indicated that 50,000 counts per minute or 4.4 per cent of the total activity was present as free steroids. A diagram of the chromatogram of this extract is presented in Figure 2. Fifty-three per cent of the extractable material was shown to be pregnanediol, 12 per cent was pregnanolone, and 35 per cent remained at the origin of the chromatogram.

β-Glucuronidase hydrolysis of another aliquot of the first 12-hour urine sample rendered 50 per cent of the total radioactivity extractable by chloroform. Chromatography of this chloroform extract showed peaks which corresponded to the same 2 compounds observed as urinary free steroids, present in about the same relative amounts (Fig. 3). Polar material was also obtained which did not move from the origin. This polar material, extracted before and after enzyme hydrolysis, could be unhydrolyzed conjugated steroid, polar unconjugated steroids, or perhaps a mixture of both types of compounds.

Metabolites in bile

Chromatograms prepared from aliquots of each of the organic extracts of lyophilized bile failed to show the presence of any free steroids. Treatment of these extracts with β-glucuronidase released about 70 per cent of the

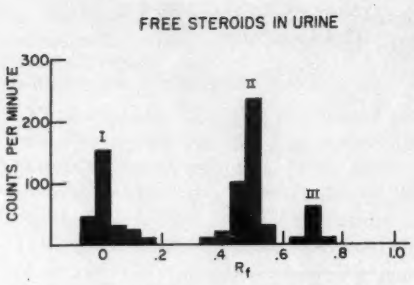

FIG. 2. Methylene chloride extraction of first 12-hour urine sample chromatographed in Bush benzene-Skellysolve C:80% methanol system.

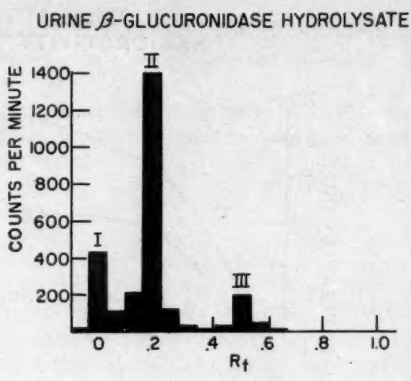

FIG. 3. Chloroform extract of first 12-hour urine sample, following treatment with β-glucuronidase, chromatographed in Bush Skellysolve C:85% methanol system.

water-soluble radioactivity, indicating that this percentage of the steroids present in bile was in the form of β-glucosiduronates. Chromatographic analysis showed that 51–60 per cent of the material liberated by enzyme hydrolysis was pregnanediol, 28–40 per cent was pregnanolone, and 9–12 per cent was unidentified polar material (Fig. 4).

Characterization of pregnanediol and pregnanolone

Results of the carrier experiments on the 2 metabolites are listed in Table 1. In each case all of the material eluted from a given chromatogram could be identified with the carrier used for its characterization. This is

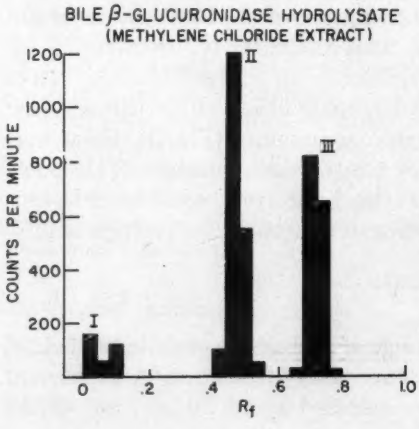

FIG. 4. Chloroform extract of first 12-hour bile sample, following treatment with β-glucuronidase, chromatographed in Bush benzene-Skellysolve:80% methanol system.

TABLE 1. IDENTITY OF METABOLITES AS DETERMINED BY CARRIER CRYSTALLIZATION

Radioactive component analyzed	Amount used (c.p.m.)	Carrier added	Solvent	Specific activity of crystalline material (c.p.m./mg.)		
				Before recrystallization	After 1st recrystallization	After 2nd recrystallization
Bile, peak II	6,800	pregnanediol (20 mg.)	methanol	342	340	
Urine, peak II	56,000	pregnanediol (20 mg.)	methanol	2370	2260	2660
Bile, peak III	4,600	pregnanolone (12.1 mg.)	heptane	396	362	378
Urine, peak III	24,000	pregnanolone (20.0 mg.)	heptane	1030	1460	

evident by the fact that there was no decrease in specific activity in any of the analyses following the first crystallization. The positive results obtained from the constant solubility tests add substantially to the identification of the 2 metabolites as pregnanediol and pregnanolone. The results of these tests are listed in Table 2.

DISCUSSION

Progesterone metabolites were excreted in both bile and urine most rapidly during the first twelve hours following the intravenous administration of a single dose of labeled hormone, and only 64 per cent of the dose could be accounted for during a 24-hour collection period. Similar studies with other steroid hormones have shown that testosterone and cortisol are eliminated to the extent of 80–90 per cent in twenty-four hours. Sandberg and Slaunwhite (13) have observed the rapid clearance of unconjugated steroids from the blood of patients given injections of progesterone-4-C^{14} and have reasoned therefrom that the relatively low levels of radioactivity in excreta are not due to a slow rate of metabolism or to strong binding of hormone or metabolites to plasma proteins. The low excretion may be ex-

TABLE 2. SOLUBILITY TEST IDENTIFYING PEAKS II AND III WITH PREGNANEDIOL AND PREGNANOLONE

A*		B†	
Equilibration time (hrs.)	Radioactivity of solution (c.p.m./ml.)	Equilibration time (hrs.)	Radioactivity of solution (c.p.m./ml.)
4	694	6	312
7	740	8	291
12	823	14	304
26	796	28	286

* Part **A:** 5 mg. of pregnanediol carrier recrystallized from a solution containing radioactive material from peak II suspended in 6.0 ml. of 80 per cent ethanol and equilibrated at 37.5° C. Measurements of dissolved radioactivity made at times indicated. After the 7-hour measurement, an additional 15-mg. portion of the same pregnanediol sample was added to the suspension.

† Part **B:** 3 mg. of pregnanolone carrier crystallized from a solution containing radioactive material from peak III suspended in 9.0 ml. of 50 per cent methanol and equilibrated at 37° C. Measurements of dissolved radioactivity made at times indicated. After the 8-hour measurement, an additional 8-mg. portion of the same pregnanolone sample was added to the suspension.

plained, in part at least, by a retention of progesterone and lipid-soluble metabolites in depot fat stores. Such retention has been observed by Kaufmann and Zander (17) following intravenous injection of the hormone into postmenopausal women. In another study, Plotz and Davis (18) estimated as much as 33 per cent of the C^{14} from progesterone-4-C^{14} is distributed in fat tissue twenty-four hours after intramuscular administration.

This report demonstrates that the biliary route of excretion is of major importance for progesterone metabolites in the human being and contrasts with observations made on the metabolism of cortisol and testosterone (19–21). Metabolites of these latter 2 steroids are eliminated mainly by way of the urine. It was found that 90 per cent of the administered doses were excreted via the urine in forty-eight hours. Approximately 10 per cent appeared in the bile in patients with T-tube drainage of the gall bladder. The excretion of administered corticosterone-4-C^{14} (22) is as rapid as that of cortisol and testosterone; however, instead of its metabolites appearing almost solely in urine, they appear in large quantities in the bile.

Solvent partition studies revealed that bile contained no detectable free steroids; however, such compounds were present in urine to the extent of 4 per cent. β-Glucuronidase hydrolysis of buffered aqueous solutions of both bile and urine demonstrated the presence of steroid β-glucosiduronates. Fifty per cent of the total radioactivity in urine was re-

leased by hydrolysis. This value agrees well with that obtained by Sandberg and Slaunwhite (13) but is somewhat lower than that reported by Bradlow and Gallagher (6). It is possible that our values represent incomplete hydrolysis, since repeated treatment with enzyme was not carried out on a given sample of urine, and our incubation times were shorter than those used by Bradlow and Gallagher. Among the free steroids in urine, as well as among those released by β-glucuronidase, pregnanediol was present in the largest amount—50 per cent of the total. The high percentage (35 per cent) of polar material remaining at the origin (Fig. 2) was not characterized chemically; however, it might represent conjugated steroids extractable into the organic solvents employed, or perhaps more polar unconjugated steroids. Pregnanolone constituted only 12 per cent of the glucosiduronate fraction of urine.

Methanol and methylene chloride extracts of lyophilized bile contained largely glucosiduronate conjugates (70 per cent). Repeated enzyme hydrolysis failed to liberate more free steroid from aqueous solutions of these extracts. This value is considerably higher than that previously reported (13). The discrepancy may be attributed to the difference in the subjects studied or to the analytical method employed. The preliminary lyophilization followed by extraction of the solid residue by organic solvents may have removed enzyme inhibitors or changed the compositions of the bile residue so that greater enzyme activity was permitted.

In bile, as in urine, the major progesterone metabolite released by β-glucuronidase was pregnanediol. However, the amount of pregnanolone was proportionately greater in bile than in urine, and in bile there was a correspondingly smaller amount of the more polar material found at the origins in the chromatograms.

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RENAL REGULATION OF PHOSPHORUS EXCRETION*

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ABSTRACT

Four male patients with apparently normal phosphorus metabolism and renal function were studied during a metabolic balance regimen and then given oral phosphorus supplements. In all cases there was an increase in the level of serum phosphorus, filtered phosphorus and urinary phosphorus. By the end of second 24-hour period, phosphorus excretion had become stabilized. Similar, but opposite responses followed withdrawal of the phosphorus supplements. Tubular response significantly affected phosphorus excretion in less than half of the studies, and in only 1 patient did it assume the major regulatory function. In several instances there were pronounced changes in the filtration rate; the tubular reabsorption, as a derived function, was more closely related to this change than to the variation in phosphorus intake. The parathyroid hormone appears to regulate renal function in response to sustained rather than transient variations in the dietary phosphorus load.

IT IS generally agreed that inorganic phosphorus enters the renal glomerular filtrate in virtually the same concentration in which it exists in the plasma. Once in the filtrate, the major portion of the phosphorus is reabsorbed by the normal renal tubule. No evidence has been presented which would suggest tubular excretion of phosphorus. Unlike potassium, the urinary phosphorus never exceeds the filtered phosphorus, even at extremely low levels of renal function (1). The amount of filtered phosphorus can be derived by multiplying the glomerular filtration rate by the serum phosphorus concentration, and the urinary phosphorus can be determined directly. The reabsorbed phosphorus can then be derived as the difference between the filtered phosphorus and the urinary phosphorus. It is apparent that urinary phosphorus can be increased by an increase in the filtration rate or in the serum phosphorus concentration, by a decrease in tubular reabsorption, or by an appropriate combination of these variables.

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Most recent studies on the regulation of phosphorus excretion have concentrated upon the role of the parathyroid hormone and its probable effect upon the tubular reabsorption of phosphorus and the glomerular filtration rate. Examination of data available in the literature suggested that variations in the serum phosphorus level after dietary phosphorus loading caused variations in phosphorus excretion, the significance of which has never been thoroughly evaluated. The present studies were designed to separate the relative importance of these factors in regulating phosphorus excretion in normal men subjected to varying phosphorus loads.

METHODS

Balance studies were conducted on 4 male patients in a metabolic research unit. The basic daily diet contained approximately 400 milligrams of phosphorus. During periods of phosphorus supplementation a mixture of sodium and potassium phosphates, buffered to pH 7.4, was administered orally at 4-hour intervals. Phosphorus in serum and urine was determined by the method of Fiske and SubbaRow (2) and creatinine in serum and urine was determined by a modification of the method of Bonsnes and Taussky (3). Daily serum values were obtained on the first 2 patients. When blood specimens were taken every six hours, it was found that there were significant diurnal variations in the serum phosphorus concentration. Therefore all calculations were based on averages of 4 values taken at 6-hour intervals. Though recognizing its shortcomings, the creatinine clearance was utilized to calculate the approximate glomerular filtration rate (GFR), as the only practical method of assessing this value for the sustained period involved. Following the convention introduced by Crawford and his associates (4) in 1950, urinary phosphorus was designated UP; the filtered phosphorus, GFP; and the amount reabsorbed by the tubules, TRP. It should be noted that the TRP of Crawford is the absolute amount of phosphorus reabsorbed per unit of time, whereas the TRP of Chambers (5) is the ratio between the amount reabsorbed and the amount filtered. Thus the TRP/GFP ratio of Crawford and the TRP of Chambers are mathematically identical. In this paper, the TRP will represent the absolute amount of phosphorus reabsorbed by the tubules as originally defined by Crawford.

RESULTS

In the first patient the period of low phosphorus intake lasted for ten days. The relationship of the GFP, TRP and UP can be observed in Figure 1. Starting the eleventh day a supplement of neutral phosphates containing 1,500 milligrams of phosphorus was administered in 6 fractional doses. It can be seen that on the eleventh day there was an abrupt increase in the serum phosphorus level, as a result of which the GFP was increased. TRP increased, rather than decreased, but proportionately less than did GFP, and there was a net increase in UP. The balance data, not charted, indicated that on the twelfth day there was a slight additional increase in UP, at which point it became stabilized until the supplement was withdrawn. By the twentieth day the serum phosphorus level had fallen, but was still elevated. GFP values were now slightly less than those for the control

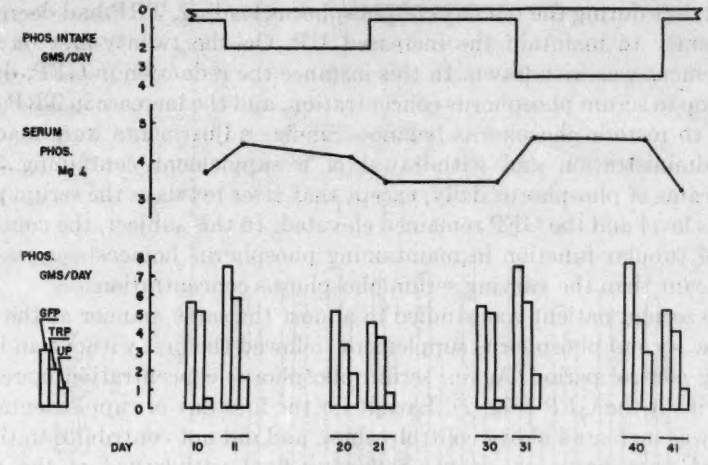

FIG. 1

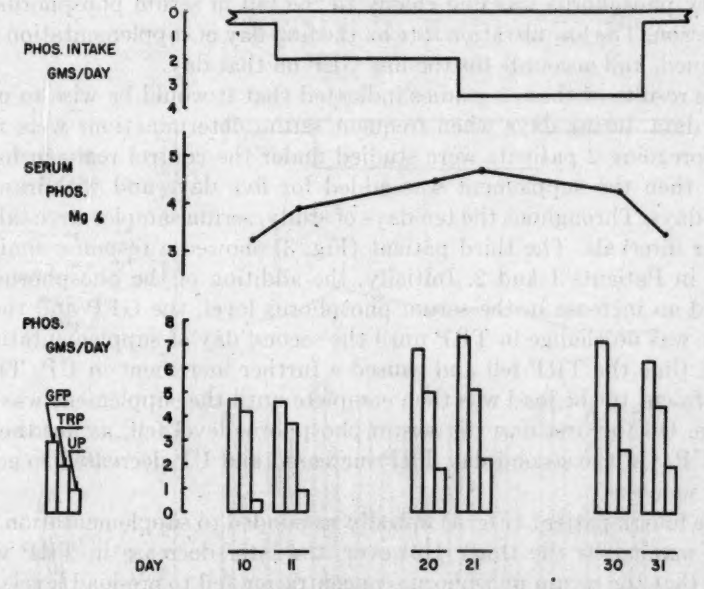

FIG. 2

FIG. 1. Phosphorus loading data—Patient 1.
FIG. 2. Phosphorus loading data—Patient 2.

(In this and subsequent figures, phosphorus intake and output are expressed as Gm. per twenty-four hours, and the serum phosphorus level as mg. per 100 ml.)

period, but during the ten days of phosphorus loading, TRP had decreased sufficiently to maintain the increased UP. On the twenty-first day the supplement was withdrawn. In this instance the reduction in GFP, due to the drop in serum phosphorus concentration, and the increase in TRP combined to restore phosphorus balance. Similar adjustments were made to the administration and withdrawal of a supplement containing 3,000 milligrams of phosphorus daily, except that after ten days the serum phosphorus level and the GFP remained elevated. In this subject, the contribution of tubular function in maintaining phosphorus homeostasis was less significant than the varying serum phosphorus concentration.

The second patient was studied in almost the same manner as the first, but the second phosphorus supplement followed the first without an intervening control period. Again, serum phosphorus concentration increased, and with it the GFP (Fig. 2). Except for the first day of supplementation, TRP was increased above control values, and did not contribute to the increased phosphorus excretion. Following final withdrawal of the phosphorus supplement, TRP fell to the initial control level; the reduction in urinary phosphorus was due chiefly to the fall in serum phosphorus concentration. The low filtration rate on the first day of supplementation is unexplained, and accounts for the low GFP on that day.

The results of these 2 studies indicated that it would be wise to obtain more data during days when frequent serum determinations were made. The foregoing 2 patients were studied under the control regimen for two days; then the supplement was added for five days and withdrawn for three days. Throughout the ten days of study, serum samples were taken at 6-hour intervals. The third patient (Fig. 3) showed a response similar to those in Patients 1 and 2. Initially, the addition of the phosphorus load caused an increase in the serum phosphorus level, the GFP and the UP. There was no change in TRP until the second day of supplementation, at which time the TRP fell and caused a further increment in UP. The adadjustment to the load was then complete until the supplement was withdrawn. On the first day the serum phosphorus level fell, as did the GFP and UP. On the second day TRP increased and UP decreased to equilibrium values.

The fourth patient (Fig. 4) initially responded to supplementation in the same manner as the third. However, the later decrease in TRP was so great that the serum phosphorus concentration fell to pre-load levels. During the period of phosphate loading there was a consistent fall in the GFR—which further reduced the GFP. On withdrawal of the load, the GFR suddenly increased above pre-load levels. Although there was a decrease in the serum phosphorus concentration, it was the increase in TRP which restored phosphorus balance. Since TRP is derived from factors

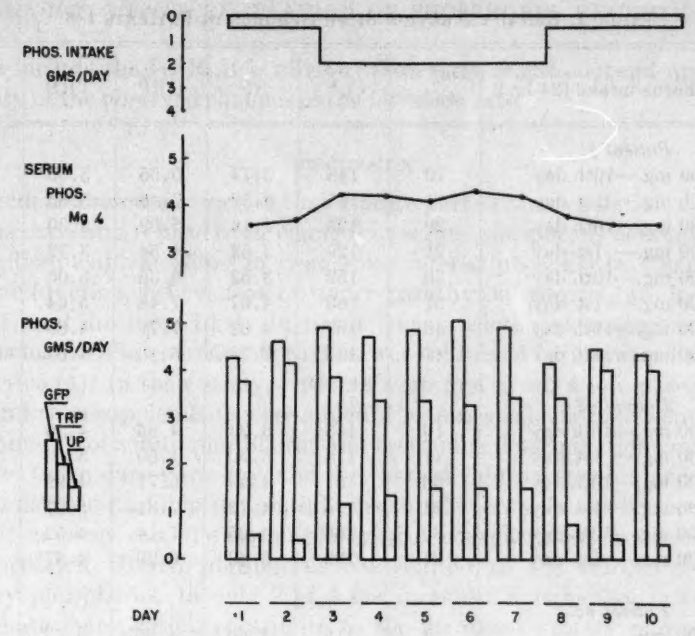

FIG. 3

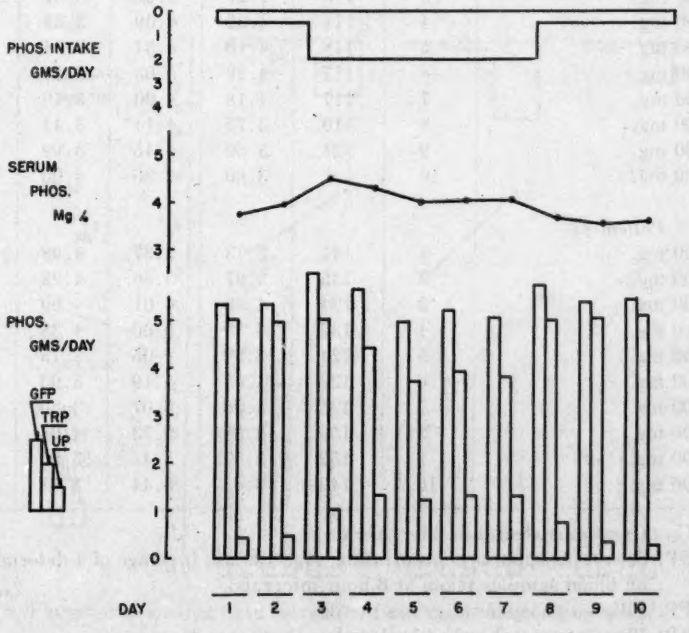

FIG. 4

FIG. 3. Phosphorus loading data—Patient 3.
FIG. 4. Phosphorus loading data—Patient 4.

TABLE 1. RENAL EXCRETION OF PHOSPHORUS IN PATIENTS 1–4

Phosphorus intake (24-hr.)	Day of study	C_{cr}*	SP	GFP	TRP	UP
Patient #1						
400 mg.—10th day	10	148	3.74	5.55	5.09	0.46
1900 mg.— 1st day	11	165	4.52	7.46	5.83	1.63
1900 mg.—10th day	20	122	4.16	5.09	3.30	1.80
400 mg.— 1st day	21	129	3.52	4.54	3.72	0.82
400 mg.—10th day	30	152	3.52	5.35	5.00	0.35
3400 mg.— 1st day	31	160	4.67	7.48	5.64	1.84
3400 mg.—10th day	40	167	4.62	7.71	4.69	3.02
400 mg.— 1st day	41	157	3.29	5.16	4.07	1.09
Patient #2						
400 mg.—10th day	10	143	3.25	4.65	4.20	0.45
1900 mg.— 1st day	11	118	3.90	4.60	3.66	0.94
1900 mg.—10th day	20	151	4.49	6.77	4.99	1.79
3400 mg.— 1st day	21	155	4.72	7.31	5.07	2.24
3400 mg.—10th day	30	168	4.25	7.14	4.52	2.62
400 mg.— 1st day	31	189	3.33	6.29	4.37	1.92
Patient #3						
400 mg.	1	118	3.60	4.25	3.77	0.48
400 mg.	2	127	3.69	4.59	4.17	0.42
1900 mg.	3	115	4.27	4.92	3.87	1.15
1900 mg.	4	111	4.22	4.69	3.33	1.36
1900 mg.	5	118	4.10	4.84	3.34	1.50
1900 mg.	6	117	4.29	5.03	3.51	1.52
1900 mg.	7	117	4.18	4.90	3.40	1.50
400 mg.	8	110	3.73	4.14	3.41	0.73
400 mg.	9	124	3.59	4.45	3.99	0.46
400 mg.	10	121	3.60	4.36	4.00	0.36
Patient #4						
400 mg.	1	144	3.73	5.37	4.98	0.39
400 mg.	2	135	3.97	5.36	4.93	0.43
1900 mg.	3	134	4.48	6.01	4.99	1.02
1900 mg.	4	132	4.30	5.66	4.38	1.28
1900 mg.	5	124	3.99	4.95	3.73	1.22
1900 mg.	6	128	4.05	5.19	3.90	1.29
1900 mg.	7	125	4.06	5.07	3.80	1.27
400 mg.	8	157	3.65	5.73	4.98	0.75
400 mg.	9	152	3.57	5.41	5.05	0.36
400 mg.	10	149	3.65	5.44	5.11	0.33

* C_{cr}: Creatinine clearance, liters per day.
SP: Serum phosphorus concentration, mg./100 ml. (average of 4 determinations on blood samples taken at 6-hour intervals).
GFP: Filtered phosphorus, grams per day.
TRP: Phosphorus reabsorbed by tubules, grams per day.
UP: Urinary phosphorus, grams per day.

which include the GFR, it is obvious that these results depend upon the validity of the observed changes in the filtration rate.

DISCUSSION

The data presented suggest that a large part of the regulation of phosphorus excretion results from changes in serum phosphorus concentration and subsequent variations in glomerular filtered phosphorus. Changes in tubular function, believed to be under parathyroid control, are less consistent, and are more likely to result from a sustained change in phosphorus load. Data comparable to ours were obtained by Crawford and his associates (4). In their study, patients were first given a low phosphorus diet and then supplements were added. Clearance calculations were based on a number of serum phosphorus and creatinine determinations made on samples taken during the day, and the charted values were obtained on the fourth day after adding the phosphorus supplements. It can be seen (Fig. 5) that in every case the serum phosphorus concentration increased, and the increased filtered phosphorus contributed to the readjustment in urinary phosphorus. In only 2 of 5 instances did a reduction in tubular phosphate reabsorption contribute to the increased urinary phosphorus, and in 1 of these this contribution was less than one-fourth of the total increase.

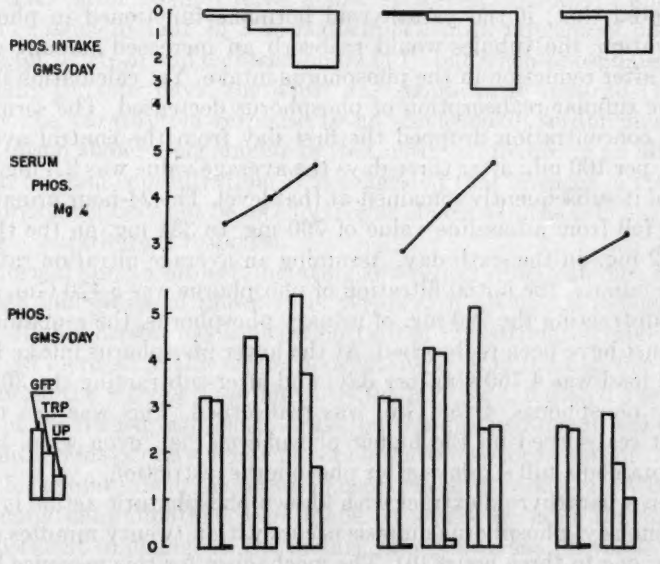

FIG. 5. Phosphorus loading data calculated from Table IV of Crawford *et al.* (4).

TABLE 2. DATA CALCULATED FROM TABLE IV OF CRAWFORD *et al.* (*J. Clin. Invest.* 29: 1448, 1950 (Ref. 4))*

Phosphorus intake (24-hr.)	C_{cr}†	SP	GFP	TRP	UP
Patient H.K.					
0.2 Gm.	93.3	3.45	3.184	3.100	0.084
0.8 Gm.	112.1	3.99	4.473	4.099	0.374
2.4 Gm.	115.7	4.68	5.415	3.718	1.697
Patient M.O.					
0.1 Gm.	112.0	2.85	3.192	3.159	0.033
0.3 Gm.	110.3	3.87	4.257	4.114	0.143
3.3 Gm.	110.0	4.75	5.225	2.650	2.620
Patient W.L.					
0.2 Gm.	97.3	2.65	2.578	2.542	0.036
1.7 Gm.	88.5	3.23	2.856	1.789	1.067

* All values corrected to 1 Sq. M. of body surface area.
† Abbreviations as for Table 1.

Chambers and his associates (5) gave 5 normal subjects a low phosphorus diet containing 430 milligrams of phosphorus daily. It would be anticipated that, if the parathyroid hormone functioned in phosphorus conservation, the tubules would reabsorb an increased amount of phosphorus after reduction in the phosphorus intake. Yet calculation indicates that the tubular reabsorption of phosphorus decreased. The serum phosphorus concentration dropped the first day from the control average of 3.8 mg. per 100 ml.; after three days the average value was 3.3 mg. per 100 ml., and it subsequently remained at that level. The 24-hour urinary phosphorus fell from a baseline value of 790 mg. to 334 mg. on the third day and 302 mg. on the sixth day. Assuming an average filtration rate of 100 ml. per minute, the initial filtration of phosphorus was 5.470 Gm. per day. After subtracting the 790 mg. of urinary phosphorus, the remaining 4.680 Gm. must have been reabsorbed. At the lower phosphorus intake level the filtered load was 4.760 Gm. per day, and after subtracting the 302 mg. of urinary phosphorus, 4.458 Gm. was reabsorbed. This was less than the amount reabsorbed on the higher phosphorus diet, even when based on data obtained a full six days after phosphorus restriction.

When a parathyroid extract with known phosphaturic action is injected intravenously, phosphorus diuresis occurs within twenty minutes and persists for one to three hours (6). The mechanism for this response has been the subject of controversy and has been attributed both to tubular rejec-

tion of phosphorus and to an increased filtration rate (7–10). Stewart and Bowen (11) raised the possibility that this response may be an artefact arising from the method of preparation of the hormone. Jahan and Pitts (12) demonstrated that there is no change in the TmP level at fourteen hours after injection of parathyroid hormone. The available data thus indicate that the phosphaturic action of parathyroid hormone is prompt but unsustained. It must be presumed that a continued effect is due to a persistent increase in the level of the hormone.

Howard, Hopkins and Connor (13) showed that the injection of calcium gluconate into normal subjects results in an average 35–40 per cent reduction of urinary phosphorus output, despite an increase in the concentration of serum phosphorus. Kyle, Schaaf and Erdman (14) made the same observation. By means of data obtained on fractional urine collections, Nordin and Fraser (15) found that phosphorus excretion fell within eight to twelve hours after initiation of the calcium infusion. It is probable that the elevation in serum calcium concentration inhibits the release of parathyroid hormone, resulting in increased tubular phosphorus reabsorption. In view of the rapid inactivation of the hormone, it appears that the response of the parathyroid gland itself must be delayed.

Crawford *et al.* (4) conducted 2 studies in which phosphates were injected intravenously. The clearance data indicated that there was a reduction in TRP after about twelve hours. However, Thompson and Hiatt found no change in TmP in 2 normal subjects after infusion of phosphate solution for fourteen and eighteen hours (16); but when phosphate was infused daily for four days, the TmP was significantly reduced (17). Apparently the parathyroid response to an elevation in serum phosphorus concentration shows a lag similar to that observed following an alteration in serum calcium concentration. It is suggested that changes in the serum phosphorus level may not be as effective as changes in the calcium level in eliciting a parathyroid response.

The normal human subject, ingesting an average American diet, excretes about 750 milligrams of phosphorus per day, or about 0.5 milligram per minute. If this person has a filtration rate of approximately 100 ml. per minute, an increase in the serum phosphorus level of 0.5 mg. per 100 ml. will increase the filtered phosphorus by 0.5 mg. per minute. If there is no change in the reabsorbed phosphorus, this 0.5-mg. increment will amount to 750 milligrams per day, and will double the phosphorus output. A variation of this magnitude in the serum phosphorus level would rarely be noted unless many determinations were made. It seems probable that much of the adjustment to change in the dietary phosphorus load is made by this mechanism. The evidence that tubular function is the primary mechanism for regulating normal phosphorus excretion in response to dietary varia-

tions in intake is not supported by the results of this study, nor by data available in the literature. Alterations in tubular function, and therefore in parathyroid control, appear to become more important in response to sustained changes in the phosphorus load.

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IODINE METABOLISM OF ENDEMIC GOITER ON THE ÅLAND ISLANDS (FINLAND)*

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ABSTRACT

Studies of the urinary excretion of radioactive iodine and stable iodine, of the concentration of protein-bound iodine in serum, and calculation of the daily production of thyroid hormones, together with clinical investigation showed that: 1) with regard to the incidence of nodularity (86 per cent), the endemic goiter on the Åland Islands is comparable with that on the Finnish mainland; 2) in normal subjects, the average 48-hour excretion of radioactive iodine was 49.3 per cent of the administered dose; 3) the daily urinary excretion of stable iodine (corrected for methodologic error) was about 49.5 μg.; 4) with increasing size of the thyroid gland there was a statistically significant decrease in the excretion of radioactive iodine; in the group with the largest goiters this excretion was only 32.2 per cent; 5) in normal subjects the calculated daily production of thyroid hormones was the equivalent of about 62.5 μg. of iodine per day when corrected for methodologic error; it rose with increasing thyroid volume to a level of about 124.5 μg. per day for the group with the largest goiters, a value which differed significantly from that for the control group.

A relatively or absolutely diminished availability of iodine to the thyroid, evidently resulting from deficient intake of iodine, plays a significant part in the development of goiter on the Åland Islands. The increased daily production of thyroid hormones in the goiter group can be explained by an increased reutilization of the iodine liberated. The pattern of iodine metabolism in this endemia is compared with pattern in other endemic regions. The relationship with the tendency towards thyrotoxicosis, regarded as a characteristic feature of the Finnish endemia, is discussed.

DURING the last few decades some investigators (1–4) have studied the endemic goiter of Finland, which has assumed considerable proportions. It is known to be most severe in the east of the country. It is characterized by a high incidence of nodules, nodular goiter being present

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in 60–90 per cent of cases (3, 5). Thyrotoxicosis is a relatively common complication, and intrathoracic multinodular goiters are not infrequent. With the adoption of modern methods of investigation, in particular the determination of protein-bound iodine in the serum and the use of radioactive iodine, it seemed to be of interest to study the iodine metabolism of endemic goiter in Finland. During the last few years 2 studies of this kind have been conducted—one in the region of Helsingfors (6, 7) and another in the east of Finland (8). A preliminary review of certain observations has recently been published (9).

The present paper deals with the situation on the Åland Islands, which are situated in the Baltic between Finland and Sweden (Fig. 1) and consist of a few larger islands and innumerable smaller islands and skerries. The ground is primarily rock, mostly "rapakivi" granite. The soil is mostly infertile, and in many places the vegetable mould is very scanty. It is difficult to dig wells, since the rock lies near the surface. Rain-water is commonly used for alimentary purposes. The inhabitants are mainly farmers, fishermen and sailors. The population is relatively homogenous; immigration has been slight.

Previous reports on the incidence of goiter on the Åland Islands have been in the nature of summaries. Statistical data have not been available until recently. According to Adlercreutz (1) in 1928, the data seemed to indicate that goiter was not infrequent. It appears from Jussila's recent statistical analysis (4) that the probable incidence of endemic goiter among school children on the Åland Islands is about 0.1 per cent, against 10 per cent or more (even 30 per cent in certain parishes (8)) in some districts on the mainland. According to personal communications of doctors working on the Åland Islands, goiter is by no means rare in adults.

In view of the fact that an increased affinity for iodine by the thyroid gland has been observed in other endemic goiter areas—in the first place by Stanbury *et al.* (10) in the Argentinian Andes, but also in the Austrian Alps (11) and in Holland (12)—it seemed pertinent to study the population of the Åland Islands from this standpoint. In the region of Helsingfors (6, 7) and especially in eastern Finland (8) it has been found that in patients with goiter the thyroid gland displays an increased affinity for iodine. In addition, the excretion of stable iodine in the urine has proved to be low in eastern Finland (8). The question was posed whether Åland constituted a counterpart to the eastern districts—an area surrounded by the sea, exhibiting a less endemic tendency. The investigation showed, however, that goiter is endemic on the Åland Islands, that it is characterized by a tendency towards formation of nodules, that the thyroid has an increased affinity for iodine, and that the daily excretion of stable iodine is decreased.

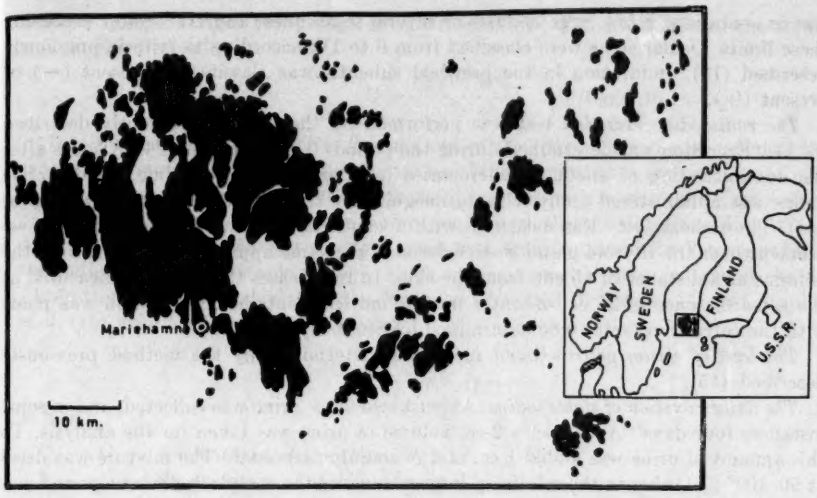

FIG. 1. The Åland Islands, Finland.

The present study is not concerned with the incidence of goiter on the Åland Islands. It deals only with certain metabolic aspects of the disease.

MATERIAL AND METHODS

One hundred and eighty subjects were investigated, but only 130 are included in the present series because the rest could not be regarded as genuine representatives of the population. The subjects who were included had lived on the Åland Islands all their lives, though some of them had spent a year or two elsewhere. Immigrants and persons who had been away for more than five successive years were omitted. The series was collected by a request, published in the local press, to appear for examination, and the study was conducted during the period May 1–22, 1956.

The investigation included a clinical examination, determination of the excretion of stable iodine (I^{127}) and radioactive iodine (I^{131}), and determination of the protein-bound iodine in serum (PBI). The daily production of hormone by the thyroid (H), and thus the daily loss of iodine from the gland, was calculated from the excretion values. In a number of cases, subcutaneous accumulations of mucopolysaccharides in the pretibial region were investigated histochemically. In some cases the biologic decay rate in the thyroid was studied by direct measurement of the radioactivity over the gland, and the iodine content of the thyroid was calculated. In 4 cases, thyroidectomy was later performed. In these, the thyroidal iodine content was chemically determined, and the histologic activity was simultaneously investigated.

Clinical examination. Relevant data with regard to family history, iodine intake and dietetic habits were carefully obtained. The thyroid was palpated and the region inspected, both at rest and during swallowing.

The thyroid gland was classified by size from 0 to III, according to the following criteria: 0 = normal; I = either diffusely enlarged up to twice the normal size or containing one or two small nodules; II = either diffusely enlarged to about three times the normal

size or containing a few large nodules or several small ones; and III = goiter exceeding these limits. Ocular signs were classified from 0 to IV, according to criteria previously described (13). Induration in the pretibial subcutis was classified as absent (−) or present (+).

The radioiodine excretion test was performed by the method previously described (6, 14). Excretion was determined during the periods 0–24 hours and 24–48 hours after the administration of about 20 microcuries (μc.) of radioactive iodide.[1] Radioactive iodine was administered orally with an insignificant amount of carrier (about 1 μg. or less). The radioactivity was measured with a scintillation counter. When direct measurement over the thyroid gland was performed, the same apparatus was used over the isthmus at a distance of 15 cm. from the skin. In these cases the dose was measured at the same distance in 25 cc. of water in a cylindrical container. Correction was made with the correction factor 0.85 determined by Stanbury *et al.* (10).

The level of serum protein-bound iodine was determined by the method previously described (15).

The daily excretion of stable iodine. At least two days' urine was collected, and in some instances four days'. As a rule, a 2-cc. volume of urine was taken for the analysis. To this amount of urine was added 1 cc. of 4 *N* sodium carbonate. The mixture was dried at 90–100° C. Owing to the relatively large volume of the sample, a 48-hour period was necessary to ensure thorough drying. The analysis was then made in the same way as in the determination of PBI.

Recovery experiments with 8 urines yielded values between 75 and 112 per cent, the mean being 86 per cent. Thus it seems probable that the values for urinary iodine obtained by this method are somewhat too low.

The iodine content of thyroid tissue. Thyroidectomy was performed in 4 cases. All these patients had nodular goiter, class III. Several biopsy specimens were examined histologically. The removed thyroid tissue was weighed. The weight of the remaining thyroid tissue was estimated to be about 5 grams. The tissue that had been removed was minced and homogenized with a fast-running tissue homogenizer of the Waring Blendor type. A sufficient volume of distilled water was added to make a thin slurry, which was further diluted with water. Total iodine was determined by adding 1 cc. of 4 *N* sodium carbonate, drying, and analyzing as in the case of PBI.

Calculations. In practically all cases the daily loss of iodine from the thyroid gland (H) was calculated by the following formula used by Riggs (16) and Stanbury *et al.* (10),

$$H = \frac{EU}{1 - U}$$

in which E = the daily excretion of stable iodine (μg.) and U = the fractional uptake of radioactive iodide by the thyroid gland. In the present investigation a 48-hour excretion test was used. Hence U had to be substituted by $1 - E^*$, which is valid, broadly speaking, when E^* = the excretion of radioactive iodine in the urine in forty-eight hours. This modification of the formula is valid as long as the thyroidal turnover rate is relatively slow. When the turnover rate is rapid, it cannot be used, since the fractional uptake will be less than $1 - E^*$ when radioactive iodine is rapidly excreted. The *biologic decay rate* was investigated in certain cases. It was found that $T\frac{1}{2}$ varied from 36.6 days to ∞ in 2 normal persons and in 8 goiter patients, whereas 2 patients with thyrotoxicosis clearly

[1] From the Radiochemical Centre, Amersham, England.

differed from the other two groups in this respect ($T\frac{1}{2} < 22$ days). Hence the formula was modified as follows:

$$H = \frac{E(1 - E^*)}{E^*} \qquad \text{(Riggs (16))}$$

In a number of patients the iodine content of the thyroid gland was calculated. As soon as the results of the radioiodine test had been computed, a second dose of radioactive iodine was given. The excretion in forty-eight hours was measured, just as in the first test. In addition, measurements were made over the thyroid gland almost daily. After seven to eleven days, two days' urine was again collected for determination of the radioactivity (and stable iodine), which was expressed as a percentage of the dose administered. In this calculation the formulas presented by Riggs (16) and Stanbury *et al.* (10) were used, according to which

$$Q_g = \frac{EU}{E^{*t}/Q_g^{*t}}$$

in which

Q_g = the iodine content of the thyroid gland,
E = the daily excretion of stable iodine (μg.),
U = the fractional uptake of radioactive iodine by the thyroid gland,
E^{*t} = the fractional excretion at the time of equilibrium, and
Q_g^{*t} = the fraction retained by the thyroid at the time of equilibrium.

In these calculations the equation $U = 1 - E^*$ was again introduced. Thus, the formula was used in the modification

$$Q_g = \frac{E(1 - E^*)}{E^{*t}/Q_g^{*t}}$$

where E^* = the excretion of radioactive iodine during the first 48-hour period.

Since the patients here concerned had goiters of moderate size, the values obtained on direct measurement of U seemed to be in keeping with the excretion values $1 - E^*$ (Table 3), which is in contrast to certain results previously obtained in Helsingfors (14).

Examination of the pretibial connective tissue. Using local conduction anesthesia, pieces of pretibial skin were taken for histologic examination from 64 subjects in such a way that the anesthetic was not allowed to infiltrate the area from which the sample was taken. In addition, a piece of the anterior tibial muscle was taken in 51 of these cases. The samples were fixed in an aqueous solution of 4 per cent basic lead subacetate, embedded in paraffin and sectioned at 10 μ. The sections were stained for the same length of time in a 1 per cent aqueous solution of toluidine blue.

RESULTS

All degrees of goiter were represented in the series. In 88 of the 103 patients (86 per cent) the goiter was nodular. The occurrence of nodules increased with increasing size of the thyroid gland.

In the controls (group 0) the *excretion of radioactive iodine* (Table 1 and Fig. 2) was 49.3 per cent (mean value) of the dose administered. With increasing size of the thyroid gland the excretion decreased. The difference between the controls and the subjects with goiter was statistically signifi-

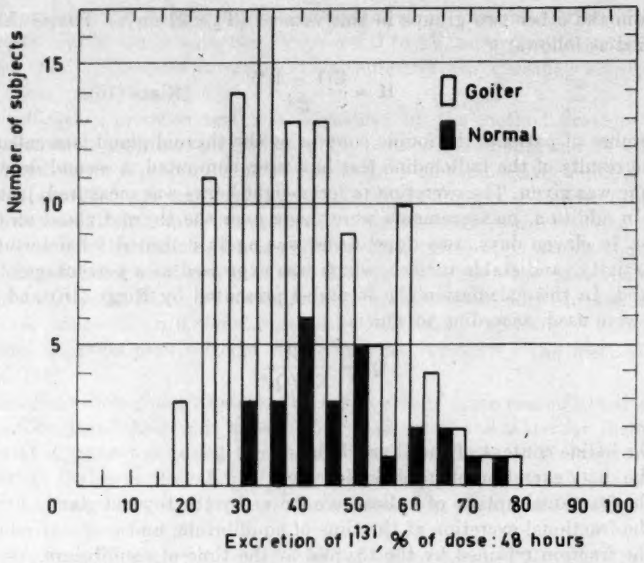

FIG. 2. Urinary excretion of radioactive iodine in goitrous and nongoitrous subjects.

cant (P 0/I <0.05; P 0/II, <0.001; P 0/III, <0.001; and P 0/I–III <0.001). The differences between groups I and II and groups I and III were also statistically significant (P I/II <0.05 and P I/III <0.002), whereas the difference between groups II and III was insignificant (P >0.1).

The excretion of stable iodine[2] was 49.1 μg. per day (mean value) in the control group (Table 1). In the group with goiter the excretion was somewhat lower. The difference, however, was not statistically significant (P 0/I–III >0.05) and the differences between the controls and the various groups with goiter were not significant either (P >0.1).

The level of serum protein-bound iodine (Table 1, mean value) was 5.04 μg. per 100 ml. in the normal group and 5.09 μg. per 100 ml. in the group with goiter. The differences between the control group and the various groups with goiter, as well as the difference between the former and the entire group with goiter, were statistically insignificant (P >0.1).

The calculated production of thyroid hormones[2] is presented in Table 1 and Figure 3. The values for H fall within a relatively wide range of variation. The mean value for the normal group was 54.4 μg. of iodine per day. An increase of H was observed with increasing size of the goiter. The differences

[2] The values in the subsequent text and the values in the tables have not been corrected for loss of iodine (15 per cent) inherent in the method of determination.

TABLE 1. DATA ON IODINE METABOLISM IN THE GOITER GROUPS

	Goiter group				
	0	I	II	III	I–III
Excretion of radioactive iodine (%dose/48 hrs.)					
Mean	49.3	43.0	36.7	32.2	39.4
Range	31.3–75.8	17.4–70.6	17.9–78.2	16.8–59.8	16.8–78.2
S.E.*	2.3	1.8	2.0	3.6	1.3
No. of subjects	27	53	38	12	103
Excretion of stable iodine (μg. per 24 hrs.)					
Mean	49.1	41.2	40.3	44.3	41.2
Range	19.0–87.9	11.6–91.5	6.0–119.3	13.8–97.9	6.0–119.3
S.E.	4.1	3.3	3.7	7.5	2.1
No. of subjects	27	52	38	12	102
Daily formation of thyroid hormones (H)† (μg. iodine per day)					
Mean	54.4	59.4	73.1	108.1	60.1
Range	8.9–175.6	13.4–181.7	11.5–278.3	25.4–391.6	11.5–391.6
S.E.	6.9	5.2	8.3	27.5	
No. of subjects	27	51	36	12	99
Level of protein-bound iodine in the serum (μg./100/ml.)					
Mean	5.04	5.00	5.12	5.38	5.09
Range	3.0–7.5	2.8–8.4	3.0–10.5	4.0–9.3	2.8–10.5
No. of subjects	27	52	38	12	102

* Standard error of the mean.

† Calculated as loss of iodine from the thyroid.

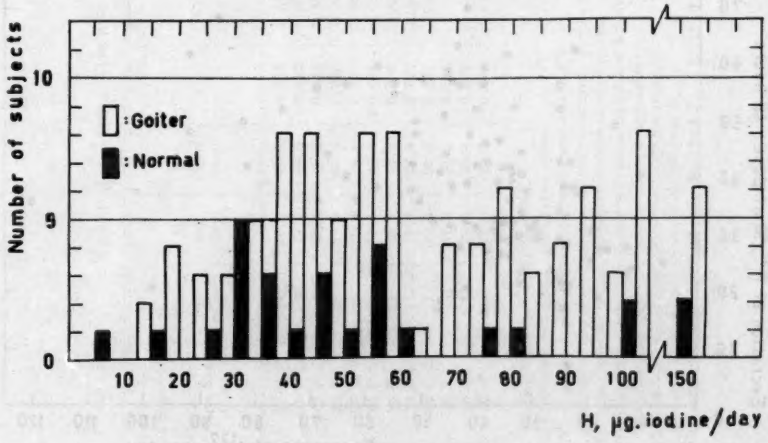

FIG. 3. Calculated daily production of thyroid hormones (as μg. of iodine) in goitrous and nongoitrous subjects.

between groups 0 and III and between groups I and III were statistically significant ($P < 0.02$ and $P < 0.01$, respectively). The mean value for the whole group with goiter (I, II and III), *i.e.*, 60.1 μg. of iodine per day, does not differ significantly from the value for the control group.

The thyroidal iodine content[2] is presented in Table 2. In 8 cases the iodine content was calculated on the basis of balance studies, and in 4 cases it was chemically determined after thyroidectomy. A considerable variation in the iodine content of the thyroid gland was observed by both methods.

The correlation between the excretion of radioactive iodine and that of stable iodine is graphically depicted in Figure 4. The excretion of stable iodine increased with increased excretion of radioactive iodine. The diagram also shows a marked variation in the individual cases.

Studies of the connective tissue. As a rule, only slight, or no pathologic changes in the connective tissue were discernible in specimens from the skin and the muscles. Thus, no definite conclusions could be drawn. In 2 cases (No. 12 of Group I, and No. 147 of Group II), there were obvious signs of myxedema (keratosis, thickening of the epidermis, ectatic small vessels with thickened walls, increased diffuse and structured metachromasia and enlargement of the interfibrillar space in the corium). In the same 2 cases the following values were obtained: serum PBI level, 4.9 and 6.1 μg. per 100 ml.; excretion of radioactive iodine, 23.5 and 23.2 per cent; excretion of stable iodine, 12.9 and 31.1 μg. per day; and calculated value

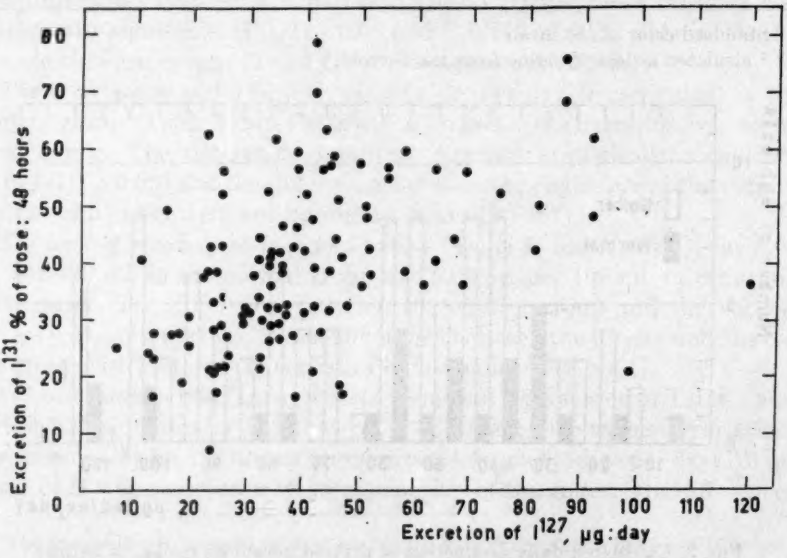

FIG. 4. Correlation between daily urinary excretions of I^{131} and I^{127}.

TABLE 2. CALCULATED AND CHEMICALLY DETERMINED THYROIDAL IODINE CONTENT†

Case No.	Subject: Age, Sex (yrs.)	Size of goiter	E (μg. /day)	E* (% of dose)	U_1	U_2	E^{st} (% of dose/day)	$Q^{st}G$ (% of dose)	H (μg. iodine /day)	PBI (μg. /100 ml.)	Q_1 (μg.)	Q_2 (μg.)	Thyroid weight (Gm.)	Chemically determined: Q (μg.)	Chemically determined: Conc. of iodine, mg./100 Gm. tissue
1	24 F	0	39.2	41.7	0.583	0.579	0.232	46.9	47.9	5.4	4,626	4,516			
19	25 F	0	79.2	66.5	0.335	0.307	0.122	25.7	39.0	3.0	5,589	5,121			
41	37 F	0	48.5	45.6	0.544	0.401	0.238	41.1	56.9	3.3	2,638	1,945			
14	30 F	II	51.0	30.0	0.700	0.756	0.590	66.5	119.0	5.1	4,025	4,347			
42	25 M	II	28.4	26.9	0.730	0.590	0.064	65.4	76.8	5.1	21,210	17,126			
43	41 F	II	39.3	43.2	0.568	0.539	0.284	49.0	52.0	3.7	3,856	3,658			
47	31 F	II	66.7	40.4	0.596	0.470	0.629	45.8	100.0	6.8	2,901	2,288			
54	32 F	II	17.3	27.8	0.722	0.844	0.125	70.0	40.4	3.5	6,997	8,179			
103	22 F	II	22.9	21.3	0.787	—	—	—	86.1	5.6	—	—	50	9,620	19.2
74	32 F	III	57.0	38.2	0.618	—	—	—	93.0	4.4	—	—	90	5,900	6.2
76	46 F	III	32.6	32.4	0.676	—	—	—	76.0	6.1	—	—	75	3,300	4.4
132	33 M	III	97.9	20.5	0.795	—	—	—	391.6	4.4	—	—	145	21,000	14.5

† E = daily excretion of stable iodine.
E* = 48-hour excretion of radioactive iodine.
U_1 = uptake of radioactive iodine calculated as 100-E^x.
U_2 = uptake of radioactive iodine directly measured over the thyroid.
E^{st} = excretion of radioactive iodine in state of equilibrium.
$Q^{st}G$ = thyroidal content of radioactive iodine in state of equilibrium.
H = calculated daily formation of thyroid hormones.
PBI = concentration of protein-bound iodine in serum.
Q_1 = thyroidal iodine content calculated from U_1.
Q_2 = thyroidal iodine content calculated from U_2.

of H, 40.9 and 104.1 μg. of iodine per day. In 1 case (No. 42), "half moon"-like accumulations of metachromatic substance were seen in transverse sections of the muscle, inside of the sarcolemma. In addition, signs of edema were observed in the skin, although the changes were not typical of myxedema. In this case the serum PBI level was 5.1 μg. per 100 ml, the excretion of radioactive iodine was 29.3 per cent, the excretion of stable iodine was 28.4 μg. per day, and the calculated value of H was 76.8 μg. of iodine per day.

A histologic study of the thyroid was made in the 4 subjects operated on (Table 2). No preoperative treatment was directed to the thyroid. In Cases 103 and 132, the histologic structure indicated complete inactivity. The epithelium was flat and the follicles were filled with colloid. They varied in size, being predominantly macrofollicular. In Cases 74 and 76 the histologic picture was less uniform. The glands were invaded by connective tissue which formed long, rather thick bundles. The thyroid tissue was predominantly microfollicular. The epithelium varied somewhat in height, being in some instances flat, in others somewhat higher, and rarely even cuboid. Here and there, lymphocyte infiltration was seen. In general, however, the picture was that of inactivity, though more active areas could be seen among the connective tissue bundles.

DISCUSSION

In normal subjects on the Åland Islands the average 48-hour excretion of radioactive iodine was found to be 49.3 per cent of the dose administered. This value is markedly lower than those reported from non-endemic areas in the United States of America (Boston, Massachusetts (17), Rochester, Minnesota (18)), but of the same magnitude as those reported from Helsingfors (6) in Finland and from Stockholm (19) and Uppsala (20) in Sweden. In eastern Finland the affinity of the thyroid for iodine is more marked (8). An obviously increased affinity for iodine has also been observed in other endemic regions, *i.e.*, the Argentinian Andes (10), Holland (12), the Austrian Alps (11) and the highlands of Venezuela (21). On the Åland Islands the excretion of radioactive iodine decreased significantly with increasing size of the goiter; in patients with group III goiters it was only 32.2 per cent.

The excretion of stable iodine differed somewhat in the various groups, but no significant differences between the groups were demonstrable. The mean values were 49.1 μg. per day in the control group, 41.2 μg. in the group with goiter, and 43.7 μg. in the whole series. The values listed in the tables are not corrected for errors in the method. When corrected for methodologic error detected in recovery experiments, the mean values

were increased by about 15 per cent, the average excretion for the whole series being thus about 49.5 μg. per day. Assuming that the proportion of thyroid hormones excreted in the feces would be between 10 and 15 per cent of the daily production of thyroid hormones, the daily intake of iodine would be between 55 and 60 μg. per day (16). If the fecal loss were one-third of the hormones produced, the intake would be about 70 μg. per day. This tallies fairly well with the figures reported by Vilkki (22) in studies from the mainland, considering that different methods of evaluation were used.

The clinical investigation showed that nodules were present in the thyroid gland in 88 of the 103 subjects with goiter, which makes a frequency of about 86 per cent. This figure is in keeping with those reported in studies from the mainland (3, 5).

The values for the excretion of radioactive iodine reported by other Scandinavian investigators are of interest (19, 20). They seem to suggest that there are endemic tendencies in certain parts of Sweden, at least. Studies regarding the extension of the endemia in Finland showed a clinical aggravation of the disease in the direction from west to east, eastern Finland being the main district of goiter. From the standpoint of iodine metabolism, too, the endemic tendencies increase in the same direction. In regard to the excretion of radioactive iodine, there is a significant difference ($P < 0.01$) between the normal group on the Åland Islands (mean 49.3 per cent) and normal subjects in Helsingfors (6) (mean 45.2 per cent). On comparison with the data presented from eastern Finland by Jussila (8) it is found, moreover, that the thyroidal affinity for iodine is more marked in the eastern districts. These observations corroborate previous results from Finland, which show that the concentration of iodine in the water is low (with a few exceptions which might be of theoretical interest) (1), that the excretion of iodine in the urine is decreased (23), and that the retention of iodine is increased in iodine tolerance tests performed in cases of nontoxic nodular goiter (24). These findings, in combination with the low iodine intake observed by Vilkki (22) on measurement of the iodine content of the Finnish food, constitute obvious indications for the introduction of general iodine prophylaxis in Finland.

In regard to the concentration of protein-bound iodine in the serum, there was no difference between normal subjects and subjects with goiter. Values of 3.5 μg. per 100 ml. or less were observed, however, in some cases (4 controls and 6 goiters—a total of 10 cases, corresponding to 10 per cent of the whole series). The cause is not readily explained. Similar observations have been made in the Andes (10), in Helsingfors (7, 14) and in eastern Finland (8). It is possible that qualitative and quantitative changes in

the relationship between the various thyroid hormones are involved. Querido *et al.* (25) showed that in the case of experimental iodine deficiency the iodine pattern in the thyroid gland changes in the direction of less iodinated products. Obviously the body attempts to produce a sufficient amount of metabolically active substances in the face of restricted iodine intake. It is possible, nevertheless, that a phase of relative hyperactivity alternates with a phase of relative hypoactivity, as suggested by Marine (26) in his classical paper on the development of nontoxic goiter. If the lower border value is reached, an inadequate uptake of iodine is bound to result (at least temporarily) in a decreased level of serum protein-bound iodine.

The increase in the daily production of thyroid hormones (H) observed in this investigation is somewhat surprising, but it may perhaps be attributed to the alternation of the 2 phases referred to in the preceding paragraph. The mean production was 54.4 μg. (62.5 μg., corrected) of iodine per day in the control group (which is in keeping with the value of 52.7 μg. reported by Stanbury *et al.* (10)) and 60.1 μg. (69.3 μg., corrected) of iodine per day in the group with goiter (56.7 μg. according to Stanbury *et al.*). The loss of iodine from the thyroid (H) increased, however, with increasing volume of the gland. In subjects with group III goiter, the value for H was 108 μg. per day, which differs significantly from the mean value for the control group. Nonetheless the excretion of stable iodine was only 44.3 μg. (51.0 μg., corrected) per day, and the serum PBI level was within normal limits. Such a pattern can hardly be explained otherwise than by assuming that the destruction of hormones and the re-utilization of iodine by the thyroid are increased. This is partly corroborated by the slow biologic decay rate. It may, perhaps, be assumed that this pattern of iodine metabolism in group III goiters is a sign of a latent tendency towards thyrotoxicosis. J. Wahlberg (3) has pointed out that the relatively frequent occurrence of thyrotoxicosis, particularly in elderly subjects with multinodular goiter, also is a characteristic feature of Finnish endemic goiter. In this investigation, no determinations of the conversion ratio, PBI^{131} level, or the rate of destruction of thyroid hormones were made—which might have furnished a clue to this problem. Further studies are required.

The pattern of iodine metabolism, as revealed by the behavior of stable and radioactive iodine in connection with the high value for H, seems to eliminate the assumption that goitrogens play a significant part in the development of endemic goiter on the Åland Islands. This conclusion is further substantiated by the fact that the biologic decay rate is relatively slow and by the histologically inactive appearance of the thyroid glands investigated. There is no resemblance between this iodine pattern and that

of the endemia produced by goitrogens described from Tasmania in Australia (27); but obviously a group investigation like the present one does not rule out the possibility that goitrogens are active within limited areas. Peltola and Vartiainen (28), for instance, reported that goitrogens are present in the milk delivered to Helsingfors from certain districts. The present results do not, either, eliminate the possibility that the occurrence of certain goitrogenic factors in small amounts may be a contributory cause, but the data available seem to indicate that the main cause is deficient iodine intake, either relative or absolute.

Factors which would prevent absorption of iodine from the intestine have not been studied in this investigation. In those cases in which simultaneous determination of excretion of radioactive iodine and direct uptake measurements were made (Table 2) the sum of the values was usually very close to 100 per cent. Even if intestinal absorption would be impaired, there is still the fact that the availability of iodine to the thyroid is deficient. In the case of the endemia in Finland, J. Wahlberg (3) in comparing the goitrous areas and the areas of calcium limestone deposits in the ground, concluded that there could be some correlations between the two. On the other hand, calcium apparently does not prevent the absorption of iodine but seems rather to act like a goitrogen, according to Taylor (29), who reported that the effect of calcium is readily overcome by an increase in the intake of iodine.

The studies of the connective tissue yielded little additional information. It is difficult, however, to evaluate the results, owing to the fact that the histochemical methods employed are inaccurate as far as a quantitative analysis is concerned. Myxedematous changes were observed in 3 patients with goiter, and metachromatic "half moons" inside of the sarcolemma in 1 of them.

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GONADAL DYSGENESIS: CASE REPORT OF A 6-YEAR-OLD BOY WITH A FALLOPIAN TUBE AND AN UNDIFFERENTIATED GONAD

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ABSTRACT

The case is reported of a 6-year-old boy with gonadal dysgenesis and a male nuclear pattern (oral smear), who underwent exploratory laparotomy. The internal genitalia on the left consisted of a Fallopian tube and a poorly differentiated gonad. On the right the gonad resembled a prepubertal testis, with an epididymis and spermatic cord, but exhibited areas of dysgenesis; there were intratubular bodies in some of the seminiferous tubules. The anatomic abnormalities associated with gonadal dysgenesis are not predictable, and can be established only by exploratory laparotomy.

THE following case of gonadal dysgenesis seems worth reporting because of unusual features which, though suggestive of true intersexuality, did not bear out this diagnosis:

S. T., a white male child, was brought to our clinic initially at the age of 19 months. At birth the attending physician told the mother the child was a male. He likewise noted an umbilical hernia in the baby. Pregnancy had been uneventful, delivery normal, and developmental pattern undisturbed. Two brothers, aged 4 and 3 years, were in good health without any recognizable congenital abnormalities. One sibling of the mother had a foot with 4 toes.

On physical examination, the child was slightly pale, and somewhat below normal in height and weight. The forehead was wide, suggestive of hypertelorism (Fig. 1). The eyes, ears and nose were normal. The 16 visible teeth were in good repair. No abnormalities of the lungs or heart were detected. There was a small umbilical hernia. The genitalia consisted of an empty scrotum and a half-inch phallic protuberance with a foreskin, glans and a urethral dimple (Fig. 2). No gonads were detected.

Laboratory studies of the blood and urine yielded normal findings. Intravenous urograms were interpreted as normal.

The urologic consultant advised the parents to bring the child back at the age of 2 years for surgical correction of the congenital hypospadias. These surgical procedures were begun at the age of 3½ years and completed two and a half years later at the age of 6 years. At that time, a note recorded the presence of a "freely movable testis in the right inguinal region." The left gonad was not detected.

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FIG. 1. Patient S. T., aged 6 years. Note prominent forehead and wide space between the eyes.

FIG. 2. Patient S. T., aged 6 years, showing physique and genital areas.

Several months later, an exploratory operation, with a view to bilateral orchidopexy, was performed. The right gonad, measuring 2.0×1.5×1.5 cm., was found in the inguinal region. A thin, white, fibrous envelope covered the lower portion but there was no covering over the brownish upper half. A spermatic cord and an epididymis were present (Fig. 3). Following removal of the biopsy specimen, the testis was easily placed in the scrotum and maintained there without tension. Structures in the left lower abdomen resembling an ovary, oviduct, round ligament and uterus were removed (Fig. 4). The pale, white gonadal mass measured 1½×1.0×1.0 cm.; several small cysts were present below the surface.

The nuclei of the cells in an oral smear (obtained postoperatively) did not exhibit any chromatin bodies, *i.e.*, they were of the male type.

The left internal genitalia. Figure 5 is a low-power photomicrograph of a section through the gonad and accompanying structures. The tubular structure at the upper portion is a typical oviduct. Figure 6 is a medium-power view of the left gonad. A thin dense fibrous capsule was continuous with connective tissue septa, the latter forming compartments of varying sizes. Many cells within the compartments appeared to be of germinal types. Where the cells were abundant, some seemed to surround masses of albuminoid material.

Within a germinal cell nest (Fig. 7), there were 2 types of cells. Peripherally, smaller, more numerous cells with fine nuclear material, distinct round nucleoli and scanty cytoplasm could be seen. Centrally, larger, less numerous cells with coarse granular chromatin, prominent irregular nucleoli, and indistinct, muddy to clear cytoplasm were noted.

FIG. 3. The right gonad and accompanying structures at operation. The open forceps lies on the white fibrous gonadal envelope; the upper portion of the gonad was devoid of such a structure. Biopsy specimen was obtained at junction point, just above tip of forceps.

Some mitotic activity was present. The general appearance was that of an embryonic, poorly differentiated gonad.

The right gonad. Microscopic examination of the right gonad revealed the general organization of a prepubertal testis. A tunica albuginea, undifferentiated interstitial

FIG. 4. Genital structures removed from the left lower abdomen. The fimbriated end of the oviduct is to the left. The gonad is below the middle third of the oviduct. The mass below the fimbriated end of the oviduct proved to be fat.

FIG. 5. Low-power photomicrograph of left internal genitalia. Oviduct is in upper portion. The gonad is ovoid and dark.

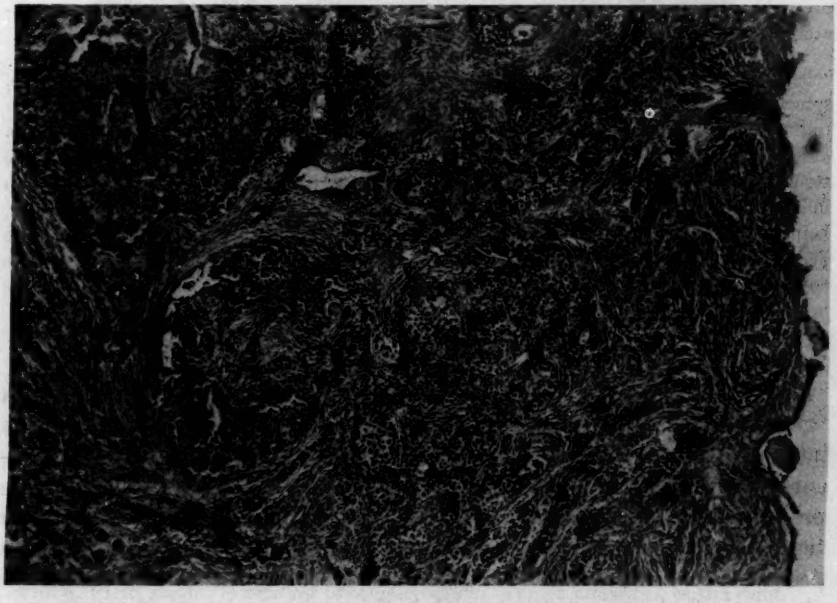

FIG. 6. Medium-power photomicrograph of the left gonad. Whorls of connective tissue forming compartments of various sizes are seen. Large cell masses are in the interior; small cell masses are towards periphery.

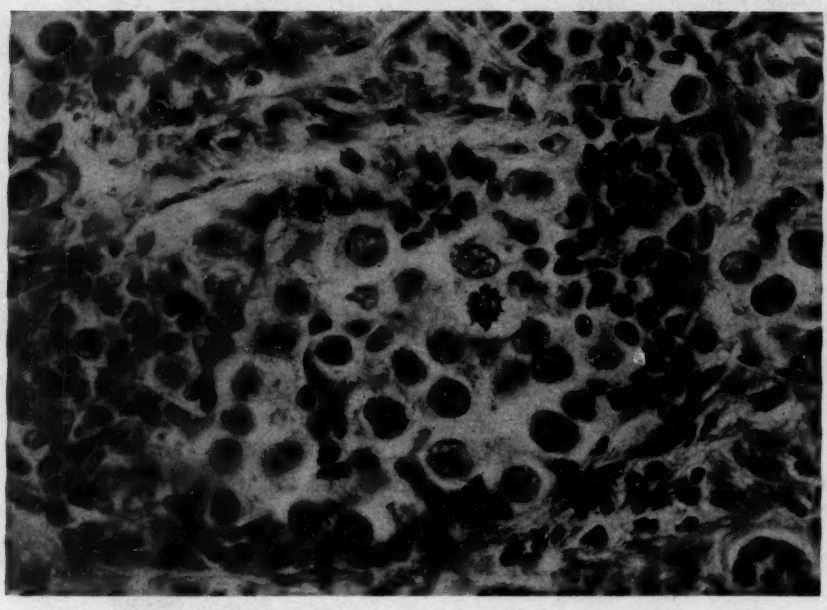

FIG. 7. High-power photomicrograph of a cell nest in left gonad. Two cell types are present (see text). Mitotic figure is seen at right center and pacyhtene form immediately above.

cells, and seminiferous tubules were present. All tubules contained sustentacular cells, some had an occasional germ cell, and a few others contained spherical structures surrounded by a second layer of sustentacular cells (Fig. 8). In some areas the tunica albuginea contained masses of germinal tissue. These were inter-connecting tubular-like structures containing 2 types of cells (Fig. 9). The sustentacular cells were quite uniform in appearance, whereas the germinal cells varied in size, configuration and number, being more numerous than in the more obviously testicular tubules. Also, there were deeply staining undifferentiated germinal cords. The interstitial elements were more compact. In general, the cellular components within the tunica were similar to those in some regions of the dysgenetic left gonad.

DISCUSSION

Grossly, the presence of a testis on the right and a Fallopian tube with a "gonad" in the broad ligament on the left suggested the possibility of true intersexuality. Microscopically, however, the left gonad was so undifferentiated that neither ovarian nor testicular organization was evident. The presence of deeply staining cords of cells was more suggestive of testicular tendencies. The negative sex chromatin findings in the oral smear did not prove that the undifferentiated gonad was an embryonic testis, because the chromatin findings in patients with both ovarian and testicular tissue can be either negative or positive.

FIG. 8

FIG. 9

FIG. 8. Right gonad; low-power photomicrograph. Tunica albuginea to the right. Seminiferous tubule, containing ovoid body, in the left center.

FIG. 9. Germinal tissue within the tunica albuginea. Germ cells are prevalent and pleomorphic.

The association of a Fallopian tube with the undifferentiated gonad can be explained by the lack of a fetal gonadal inductor. Jost (1) demonstrated that in the absence of a fetal testis the Fallopian tube would develop. Theoretically, then, the undifferentiated left gonad in this boy did not exert a localized male inductor action. The testis on the right side must have exerted its embryonic inductor action, since the Müllerian tract was absent and the testis has an epididymis and spermatic cord (Wolffian structures).

The undifferentiated character of the left gonad would justify its classification as a germinoma or gonadoblastoma. Ultimately it might have become a malignant seminoma. The right gonad, although having some of the attributes of a prepubertal testis, also had areas of dysgenesis. There were intratubular bodies in some of the seminiferous tubules.

Several types of intratubular bodies in human testes have been reported. Oiye (2) described calcareous concretions in 6 adult testes, 4 of which were devoid of spermatogenesis. By staining and chemical reactions, all of the concretions were shown to contain calcium, whereas amyloid reactions were negative. Blumensaat (3) described intratubular bodies in 6 prepubertal testes out of 52 that were studied. Both of these authors assumed that the bodies originated from intratubular cells or cell clusters. Blumensaat described nuclear structures and believed the bodies represented large spermatogonia. Sniffen (4) pictured "colloid substance" within the tubule of a 6-month-old infant. He further stated that germ cells lying centrally within the cords were undergoing degeneration. Sohval (5) depicted "colloid like bodies" in the testes of 6 patients. Four of these were prepubertal (3 being abdominal). The 2 adult testes were inguinal and contained nodules of embryonal tubules indicative of dysgenesis.

It is thus apparent that intratubular bodies are consistently associated with immature or defective seminal tubules. The acellular calcified bodies may represent late stages of degeneration. Only 1 published illustration (Blumensaat's patient 1102/27, a 5¾-year-old boy with inguinal testes) has any close resemblance to the intratubular body shown in Figure 8 of this paper or in the case reported previously. Blumensaat emphasizes the corona of sustentacular cells commonly observed in immature testicular cords or tubules.

The coronal arrangement of the immature Sertoli cells seems to be a special response to the intratubular body, as this arrangement is not evident around the aggregates of desquamated cells commonly seen in the tubules of the immature testis (Fig. 8). Because we first observed these intratubular bodies in the gonadal tubules of a boy with chromatin-positive findings in the oral smear, we considered them as oocytes and enumerated our reasons (6). Further observations, using special staining

techniques, will be necessary in order to study the origin and nature of these intratubular bodies. If their occurrence is limited to cases in which there is evidence of gonadal dysgenesis, they may represent germ cells that develop atypically in an abnormal environment (Fig. 10).

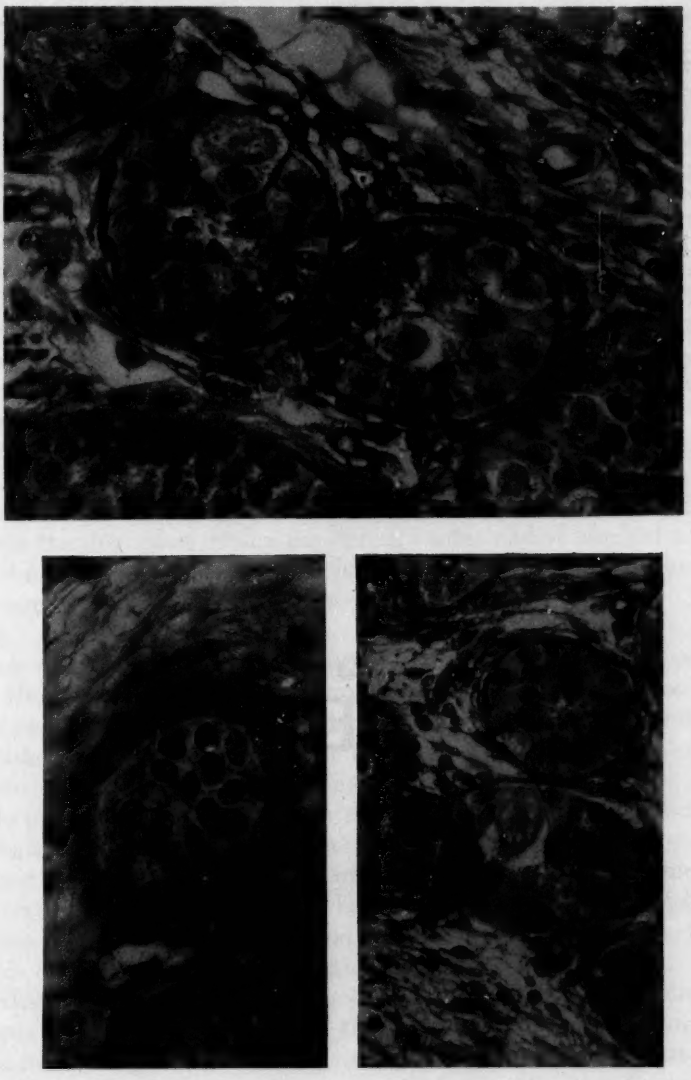

FIG. 10. Germ cells exhibiting bizarre types of proliferative activity. These tubules were all in areas of dysgenesis.

Even though the results of the chromatin test may indicate the intended genetic sex of the subject, the anatomic deviations associated with dysgenesis are not predictable. An exploratory laparotomy remains the only procedure which will establish the anatomic nature of the internal genital organs.

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Editorial

A REVIEW OF "AUTO-IMMUNIZATION" IN HASHIMOTO'S DISEASE

PROBABLY the most interesting development in the field of thyroidology since the discovery of triiodothyronine is the finding of an antibody to thyroglobulin in the serum of certain patients with struma lymphomatosa (Hashimoto's disease). The discovery was the outcome of two independent lines of research, one American and one British.

In 1927 Hektoen and associates (1) in Philadelphia reported that saline extracts of thyroid glands ("thyroglobulin") had distinctive immunologic properties. Precipitating antibodies developed in the serum of a rabbit that received injections of the thyroid extract of another species. In that they would react with thyroid extracts only, the precipitins were "organ-specific." However, they were not "species-specific," for serum from an animal sensitized to the extract of one mammal's thyroid reacted equally well to the thyroid extract of another mammal. Hektoen's group studied not only the dog, goat, guinea pig, horse, rabbit and sheep, but also the bear, deer, racoon and zebra. The lack of specificity of precipitins within mammalian species has been generally (2–4) but not wholly (5) confirmed.

Three decades later Witebsky and Rose (6) in Buffalo, having corroborated Hektoen's observations, attempted (7) to produce iso-antibodies by sensitizing one rabbit to the thyroglobulin of another rabbit. This they accomplished by improvements in the technics of sensitizing the rabbit and of measuring its antibody titer. The sensitization was achieved by mixing the thyroid extract with Freund's adjuvant (8)—mineral oil and killed mycobacteria—and injecting the mixture into the animal's footpad. The resulting enhanced and prolonged formation of antibodies was measured by the very sensitive "tanned-cell hemagglutination" method of Boyden (9). The effectiveness of this method depends upon the preliminary exposure of erythrocytes to a weak solution of tannic acid.

Next, Rose and Witebsky (7) challenged one of the most fundamental concepts of immunology, namely, that an animal cannot produce antibodies (auto-antibodies) to any of its own proteins. Actually, rare exceptions had already been noted: auto-antibodies to the lens, to casein, to spermatozoa, and to certain extracts of the brain had been produced. To

test the immunologic dogma with the protein thyroglobulin, rabbits were given injections of extracts of their own thyroid glands. Antibodies often developed. Studies on dogs and guinea pigs confirmed the possibility of producing auto-antibodies to thyroglobulin (10).

However, might not the thyroglobulin have been denatured during its extraction from thyroid glands? If so, the animals were merely reacting to foreign (denatured) protein. The production of true auto-antibodies, Witebsky's group felt, should be accompanied by damage to the animal's own, intact thyroid gland. To test this hypothesis (10, 11), one lobe of a rabbit's thyroid gland was removed. From it, thyroglobulin was prepared and injected into the same animal's footpad. After an interval of about three weeks, circulating antibodies were found in most of the rabbits tested. A month later the remaining lobe of the thyroid gland was removed. Published photomicrographs (7, 10–14) of sections of the animals' thyroid glands were suggestive of those of tissues as seen in Hashimoto's disease. This surprising finding led to a search for antibodies to thyroglobulin in patients with this disease. In 1957 the Buffalo group reported (10) that 12 of 18 patients had such antibodies.

Parenthetically, Witebsky's group (12) recently divided the chronic lymphadenoid thyroiditides into 2 categories—Hashimoto's disease and "chronic nonspecific thyroiditis." Serum antibodies were demonstrable only in the latter group. Whether the division actually was based on histologic rather than on immunologic data is not completely clear; the categorization is somewhat at odds with Hashimoto's original description (15).

The second line of investigation is of more recent origin; it began in 1953 with an observation by Fromm, Lascano, Bur and Escalante, in Buenos Aires (16). Four patients with Hashimoto's disease were found to have significantly elevated values for serum gamma globulin. Others have since noted this electrophoretic change, and also that results of the "colloidal gold," "thymol turbidity" and "cephalin flocculation" tests frequently were positive in the presence of this disease (17–20).

On the basis of these observations the London investigators, Roitt, Doniach and Campbell (21), in 1956 suggested that the serum abnormalities might represent an immunologic response. Later in the same year, Roitt and associates (22) detected precipitins in the serum of 7 of 9 patients with Hashimoto's disease. These antigen-antibody precipitates formed when a layer of human "thyroglobulin" was placed over a layer of a patient's serum.

The result of the precipitin test may be negative when the concentration of either antigen (thyroglobulin) or antibody (serum gamma globulin (21)) is excessive, even though both are present. However, by allowing the antigen and antibody to diffuse toward each other through a gel (23, 24), a

sharply defined precipitate forms at the zone of optimal concentrations. The diffusion may be horizontal in a Petri dish (Ouchterlony plate (25)) or vertical in a test tube (26).

Using the agar technic, Doniach and Roitt (27) demonstrated precipitins to purified thyroglobulin (28) in the serum of 109 of 144 patients with Hashimoto's disease. The precipitin titers tended to be highest in untreated patients. The titers generally were lower in those treated with desiccated thyroid preparations. After thyroidectomy, or after myxedema had set in, the precipitin often disappeared from the serum (29).

In Table 1 it can be seen that antibodies to thyroglobulin are not limited to patients with Hashimoto's disease. Precipitating or hemagglutination antibodies also have been found in significant proportions in subacute thyroiditis and in primary myxedema. A puzzling discrepancy between the results of the 2 methods was found in the presence of thyrotoxicosis by Roitt and Doniach; hemagglutinins frequently were found and precipitins rarely were found.

The 2 lines of investigation have converged: on the one hand, animal research succeeded in yielding auto-antibodies to thyroglobulin which led to a comparable finding in patients with Hashimoto's disease; on the other hand, elevated values for gamma globulin in these patients suggested an immunologic abnormality which was confirmed.

Does this mean that the pathogenesis of struma lymphomatosa has been

TABLE 1. AUTO-ANTIBODIES (P-PRECIPITINS, H-HEMAGGLUTININS, C-COMPLEMENT FIXATION) IN VARIOUS DISEASES OF THE THYROID GLAND, AS WELL AS IN CONTROLS*

	Doniach & Roitt (27) P	Goudie *et al.* (30) P	Trotter *et al.* (31) P	Roitt & Doniach (29) P	Paine *et al.* (12) H	Roitt & Doniach (32)		
						P	H	C
Hashimoto's disease; chronic thyroiditis	109/144	20/32	12/24	66/107	14/32	15/85	80/85	80/85
Subacute (De Quervain's) thyroiditis	5/9			4/6	2/4	7/18	12/18	11/18
Nongoitrous myxedema	6/23	4/28	3/9	6/27		12/49	24/49	24/49
Thyrotoxicosis	0/68	3/120	0/10	0/68	1/3	3/102	65/102	46/102
Nontoxic simple and nodular goiters		0/17	0/18	0/103	1/22	0/102	38/102	14/102
Thyroidal carcinoma		0/3			1/6	0/18	4/18	2/18
Riedel's struma				0/1	1/3			
Patients without overt disease of the thyroid		0/133	0/21		0/120			

* The results are expressed as the number of patients with antibodies among the total number of patients studied.

solved (33)? Before any new theory is accepted too readily, it is well to remember that other speculations have been fostered in the past: overstimulation of the thyroid gland by the thyrotropin of the pituitary; adrenal insufficiency; infection, as the term "thyroiditis" suggests; and—one of the more interesting proposals—hypovitaminosis as the result of the eating of "American white flour" (34).

An attractive hypothesis was offered by Roitt and Doniach (32); it is based on the newly discovered immunologic findings. They postulated that initially some mechanism must permit the localized release of thyroglobulin (colloid) from the thyroid follicles. Once the disease has developed, this leakage can be demonstrated (White (35)). Fluorescein-labeled antibody from the serum of a patient with Hashimoto's disease showed localized fluorescence within the colloid of slices of thyroid substance taken from the same patient. In addition, streaks of fluorescence projected out into the interfollicular spaces. Furthermore, iodinated compounds have been recovered from the serum of patients with struma lymphomatosa (36, 37); these nonthyroxine substances conceivably could be the thyroglobulin escaping from the thyroid gland.

The first escape of thyroidal colloid from its natural, perhaps "immunologic-proof," habitat requires explanation. Some disease state could be the answer. Might thyrotoxicosis be the precursor of Hashimoto's disease? Both diseases occur primarily in women, both exhibit lymphoid changes (38), and antibodies to thyroglobulin frequently are found in both. However, on clinical grounds, Graves' disease rarely develops into Hashimoto's disease.

Might subacute (De Quervain's) thyroiditis be the precursor? Again, the distribution according to sex and the histologic and immunologic findings are compatible in the 2 diseases. But, as with hyperthyroidism, a history of subacute thyroiditis rarely is elicited from the patient with Hashimoto's disease. If it were possible for unrecognized instances of subacute thyroiditis to occur commonly, as for example a complication of mumps (39), this no longer would be a valid objection.

To return to the immunologic hypothesis: by some means, thyroglobulin escapes from the follicle. A lymphadenoid response quickly develops. The histologic picture resembles that seen in experimental animals given large doses of radioiodine (40). The reaction must progress, according to Thomson (41), until the thyroid gland is infiltrated by lymphoid tissue with follicle formation, until a diffuse infiltration by lymphocytes and plasma cells takes place, and until eosinophilic degeneration of the epithelium has occurred, before the result of the serum precipitin test becomes positive. Lesser degrees of lymphoid infiltration are found in the thyroid glands of 15 per cent of older women without clinical disease of that gland, in about

40 per cent of nontoxic nodular goiters, and in some 80 per cent of thyrotoxic goiters (32).

It is believed that plasma cells, which accompany the lymphocytes into the irritated thyroid gland, produce antibodies to the thyroglobulin. A chain reaction has now been set in motion: colloid escape, plasma-cell reaction, antibody formation, antigen-antibody precipitation within thyroidal follicles, additional epithelial damage, increased leakage of colloid and so on. Since Hashimoto's disease does not develop in most patients with thyrotoxicosis, despite the lymphoid infiltration, the immunologic reaction must be considered to be reversible, according to this hypothesis.

The final stage of Hashimoto's disease apparently reflects "antigen-exhaustion"; there are no remaining undamaged thyroidal follicles. The relentless cycle, perhaps stimulated by thyrotropin (20), has driven the patient into a state of myxedema. Since a number of patients with "primary" myxedema have circulating antibodies to thyroglobulin, perhaps they have unknowingly gone through the thyroiditic cycle.

Certain questions are not answered by the auto-antibody theory.

In the reported series (Table 1) approximately a third of the patients exhibited no circulating precipitins or hemagglutinins. Some of these determinations were made postoperatively and some patients had reached the stage of myxedema, but in some cases was not the disease still active? If so, would such patients have to be classified differently from the others? Can any correlation, qualitative or quantitative, be shown between histologic pattern and antibody titer? In the case of the auto-immunized rabbits such a correlation seemed to hold, but it did not necessarily do so in dogs and guinea pigs exposed to their own thyroid extracts (13).

Are the results of Witebsky's animal studies and the findings in patients actually comparable? The animal antibody did not show mammalian species specificity. The one found by Doniach and Roitt (27) in patients was completely specific when exposed to thyroglobulin from the rabbit, rat, sheep, hog, cow or horse. However, thyroglobulin from the Rhesus monkey or the chimpanzee was able to cross-react with the human antibody (32). Despite this apparent similarity between human and monkey thyroid, injection of a large volume of serum from a patient with Hashimoto's disease—serum with an antibody concentration of 0.5 Gm. per 100 ml.—into a monkey failed to cause histologic change in its thyroid gland, and there was no thyroid fixation of the antibody. Does this mean that the success of Witebsky's group in producing antibodies depended on the adjuvant with which the thyroglobulin was mixed? In other words, must there perhaps be some cytotoxic agent as well as an antibody to induce thyroiditis? Or is the artificially produced antibody in animals simply different from that found in man? Without the aid of an adjuvant, Lilien

(42) was able partially to destroy rat thyroid glands by the intraperitoneal administration of serum from rabbits sensitized to extracts of rat thyroid glands.

The most curious finding in the immunology of struma lymphomatosa has to do with an antibody which is not directly related to the one which causes precipitation or hemagglutination. This antibody "fixes" complement and is measured by superimposing a hemolytic reaction, which also requires complement, on the thyroglobulin-antibody reaction. If a patient's serum contains complement-fixing antibodies, the secondary hemolytic reaction cannot occur; in the absence of antibodies the erythrocytes are completely hemolyzed. This is the standard complement-fixation test.

In the serum of patients with Hashimoto's disease, antibodies of the tanned-cell agglutination type and of the complement-fixing type usually are present (Table 1) (29–31). This might suggest that the antibodies actually are one and the same. However, in the other thyroidal diseases Roitt and Doniach (32) commonly found only one or the other antibody to be present; this was particularly true in patients with thyrotoxicosis. On the other hand, it seems likely that the same antibody accounts for the hemagglutinin and precipitin reactions. Roitt and Doniach (32) found that results of the 2 tests were closely parallel in the various types of thyroidal disease, despite the much greater sensitivity of the hemagglutination test.

It is in the antigenic half of the complement-fixing reaction that confusion arises. This antigen is not found at all, or only in minute amounts, in patients with normal or nontoxic thyroid glands; it is almost invariably present in thyrotoxic glands (31). However, the antigen to precipitin and hemagglutinin antibodies is found in normal and thyrotoxic glands alike. It has been shown (31) that the antigen to the complement-fixing antibody lies in the acinar cells, but the antigen to precipitins is in the colloid; the 2 are separable by ultracentrifugation, once they have been extracted from a toxic gland. In the serum, the 2 antibodies are distinguished by first precipitating the precipitin with thyroglobulin; the complement-fixing antibody remains unchanged (32).

Why should patients with Hashimoto's disease regularly exhibit in their serum complement-fixing antibodies to an antigen obtainable only from the gland of a thyrotoxic patient? Patients with Graves' disease also have this antibody frequently—62 per cent in Goudie's series (30) and 45 per cent in Roitt and Doniach's (32). What is its significance?

The immunologic approach to Hashimoto's disease has stimulated interest not only among thyroidologists, but among immunologists generally, and among physicians interested in diseases of other tissues of the body which might conceivably enter into auto-immune reactions. Some of the diseases which bear certain resemblances are autohemolytic anemias,

allergic encephalomyelitis, rheumatoid arthritis, disseminated lupus erythematosus, macroglobulinemic states, and infectious hepatitis (43). In Gajdusek's thorough analysis (44) of possible auto-immune diseases, 1 patient each with "lupoid" hepatitis, chronic biliary cirrhosis, disseminated lupus erythematosus, and macroglobulinemia exhibited high complement-fixing titers to thyroid extracts; these were exceptional, however. Auto-immune diseases of other organs may be associated with similar thyroidal diseases—liver (19), kidney (45), adrenal glands (46) and Paget's disease of bone (45, 47). It is apparent that the immunologic dogma of "horror autotoxicus" is undergoing thorough scrutiny.

Whether the finding of circulating antibodies to thyroglobulin in the presence of Hashimoto's disease is related to the cause of the disease, or is only the result of a still-unexplained pathologic process, a new avenue of research has been opened. It will have been profitable even if the only immediate outcome is the development of a practical, accurate laboratory test for the evaluation of certain diseases of the thyroid gland.

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Letters to the Editor

THYROID FUNCTION OF EUTHYROID PATIENTS DURING AND AFTER TREATMENT WITH TRIIODOTHYRONINE

To the Editor:

The evaluation of thyroid function shortly after treatment of patients with triiodothyronine (T_3) is complicated because of changes brought about by the drug itself. These include increased metabolic rate and depression of both thyroidal radioactive iodine (I^{131}) uptake and serum protein-bound iodine concentration (PBI). Therefore, the state of a patient's inherent thyroid function can be studied only after T_3 has been witheld for a suitable period. Because T_3 is now used in the treatment of several states, including actual or supposed hypothyroidism, the length of this period should be known. This report describes the thyroid function of normal subjects after four weeks of treatment with 0.15–0.20 mg. of T_3 per day. In every case, there was an immediate phase of thyroidal "rebound" hyperactivity during which the I^{131} uptake was more complete than normal. This was followed, in the third and fourth weeks after T_3 was discontinued, by a gradual return of normal function.

CLINICAL MATERIAL AND METHODS

The study was conducted on 4 male and 2 female patients, 17 to 38 years old, who were hospitalized because of moderately far advanced pulmonary tuberculosis.[1] Otherwise, they were all in normal health, and were free of evidence of abnormal thyroid function. Throughout the study they were treated with isoniazid in a dosage of 5 mg. per Kg. daily and 1 Gm. of streptomycin three times a week. They were given a general diet and were ambulatory. As part of an entirely unrelated investigation, to be reported elsewhere, they were given 0.15–0.20 mg. of triiodothyronine[2] by mouth daily during alternate months. Thus, in the same patient, one or two periods each lasting four weeks during which T_3 was given could be contrasted with the intervening control periods. Such a course of treatment was observed 9 times in the 6 patients studied.

[1] These patients were hospitalized on the tuberculosis service of the Rockefeller Institute Hospital. It is a pleasure to acknowledge the cooperation of Drs. Diethelm Böhme, Suydam Osterhout, Russell W. Schaedler, Harold J. Simon and James G. Hirsch.

[2] L-Triiodothyronine (Cytomel®) was generously supplied by Smith, Kline and French Laboratories, Philadelphia, Pa.

Thyroidal I^{131} uptake[3] was measured twenty-four hours after the oral administration of 1.0–6.5 μc. of I^{131}. The serum PBI concentration was measured by the method of Barker (1). The rates of disappearance from the serum of both T_3 and thyroxine (T_4) were measured in 1 patient during the second half of a period of treatment with T_3. A dose of 0.6 μg. of T_3 labeled with 51 μc. of I^{131} (and at a later time 1.5 μg. of thyroxine labeled with 35 μc. of I^{131}) was injected by vein. The serum radioactivity was measured during the following seven to ten days. Samples of serum drawn three and six days after I^{131}-labeled thyroxine was given were analyzed by conventional methods to show that essentially all of the serum radioactivity was protein-bound, and that it migrated during starch block electrophoresis with the typical mobility of thyroxine-binding protein. After extraction with butanol the serum radioactivity migrated during paper chromatography with the typical mobility of thyroxine.

RESULTS

The usual changes of thyroid function which result from administration to triiodothyronine were observed in each subject. During the first week of

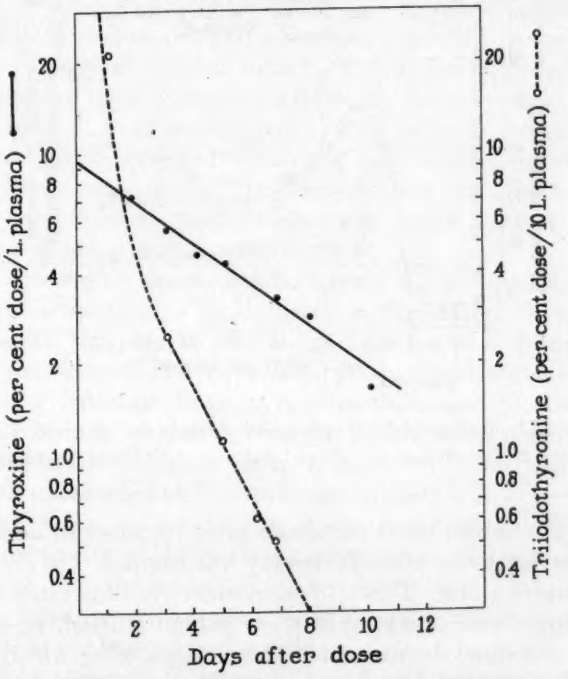

FIG. 1. Rates of disappearance of serum radioactivity after intravenous administration of I^{131}-labeled thyroxine and triiodothyronine to a patient during oral treatment with 0.2 mg. of triiodothyronine daily.

[3] I^{131} and I^{131}-labeled thyroxine and triiodothyronine were obtained from Abbott Laboratories, Oak Ridge, Tennessee, under allocation from the U. S. Atomic Energy Commission.

treatment, nervousness, tremor, tachycardia and a marked increase in the basal metabolic rate were regularly observed. The level of serum PBI dropped and thyroidal I^{131} uptake fell to, and remained at, low levels throughout the course of treatment. The rates of disappearance of intravenously administered I^{131}-labeled triiodothyronine and thyroxine in 1 patient during the third and fourth weeks of treatment are shown in Figure 1. The disappearance rate for T_3 indicated a half-time of 1.6 days and that for T_4, 4.4 days.

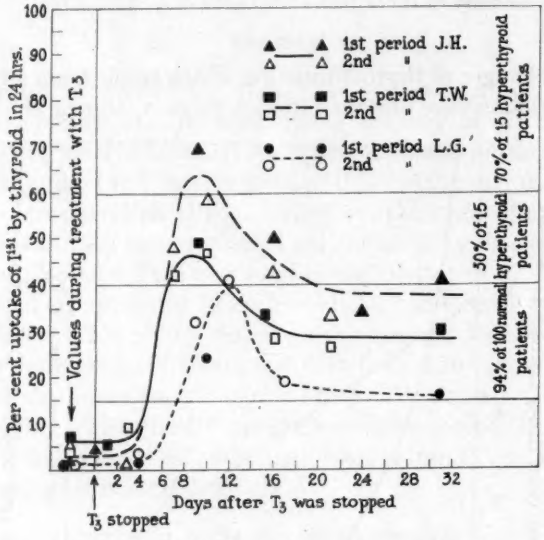
Per cent uptake of I131 by thyroid in 24 hrs.
Values during treatment with T3
1st period J.H.
2nd "
1st period T.W.
2nd "
1st period L.G.
2nd "
70% of 15 hyperthyroid patients
30% of 15 hyperthyroid patients
94% of 100 normal patients
Days after T3 was stopped
T3 stopped

FIG. 2. The 24-hour thyroidal I^{131} uptakes of patients during the first month after each of two 30-day periods of treatment with triiodothyronine.

The symptoms and basal metabolic rates returned to normal between the third and sixth day after T_3 therapy was stopped. The changes in thyroidal I^{131} uptake after T_3 was discontinued are illustrated in Figure 2. Similar changes were observed in every patient studied. In each case the I^{131} uptake remained depressed for several days, after which there was a period during which I^{131} uptake was much more complete than normal. Normal function gradually returned during the third and fourth week after T_3 was discontinued. Serum PBI concentrations in 2 patients are listed in Table 1. The expected fall in the PBI level during treatment was observed. PBI concentration gradually returned to normal during the first month after treatment was stopped.

TABLE 1. PROTEIN-BOUND IODINE CONCENTRATIONS IN THE SERUM OF 2 PATIENTS AFTER DISCONTINUATION OF TREATMENT WITH TRIIODOTHYRONINE (T_3)

	PBI ($\mu g.$/100 ml.)	
	Patient J.R.	Patient R.W.
At the end of 30 days of treatment with T_3	1.9	2.2
Days after discontinuation of treatment:		
8	2.6	2.0
15	4.6	3.6
24	7.2	5.9

DISCUSSION

It is well known that serum PBI concentration falls to a low level during treatment of normal subjects with T_3. This is partly the result of depressed synthesis of thyroid hormone. However, the very rapid disappearance of I^{131}-labeled T_4 from the serum of 1 of our patients during treatment with T_3 suggests that increased utilization may also be instrumental in lowering the PBI concentration. The disappearance rates, both for T_4 and T_3, were much more rapid than those seen in normal subjects and quite similar to those of hyperthyroid patients (2–4).

Rebound increased I^{131} uptake has been observed infrequently during or after treatment with T_4 (5, 6) and during recovery from thyroiditis (7). However, rebound thyroidal hyperactivity of the degree and frequency observed in patients treated with T_3 has been produced otherwise only by administration of goitrogenic drugs such as methimazole (8). During such treatment, pituitary release of thyrotropin is stimulated and the thyroid becomes hyperplastic. Rebound occurs immediately after treatment because synthesis of organic iodide is no longer inhibited. In contrast, during administration of T_3, its localization in the hypothalamus presumably results in the depression of the pituitary output of thyrotropin (9, 10). The concentration of T_4 in the serum falls to low levels, so that when T_3 is discontinued the extrathyroidal organic iodide space is rapidly depleted and acute stimulation of the pituitary results.

From the data presented, it appears that several parameters of thyroid function may be affected for about three weeks after discontinuation of treatment with triiodothyronine.

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CONCERNING THE REVERSIBILITY OF OSTEOPOROSIS

To the Editor:

The case presented in Dr. Robert F. Skeel's paper, "The Reversibility of Osteoporosis in Cushing's Disease" (1), closely parallels a case presented by Albright (2). This was one of the signal observations that led Albright to contend that osteoporosis, although it could be arrested, was probably not reversible.

The patient with Cushing's syndrome described by Albright was 11 years old in 1943, at which time radiographs showed severe osteoporosis. Fifteen months after remission of the disease, new and normal bone was evident along the superior and inferior borders of the osteoporotic vertebrae, as shown in radiographs taken in 1945 and as diagrammed in the report of this case. The osteoporotic bone remained visible at the core of the vertebrae, and was still evident in films made in 1955. In the radiographs presented by Skeels, the same phenomenon is present: bone of decreased density is clearly visible at the center of each vertebra in Figure 5; the bone of normal density is entirely new bone.

"Reversibility" of osteoporosis in the long bones of children may appear to occur after eradication of an etiologic agent. The bone is, in fact, new bone as growth and remodeling occur. The vertebrae, where remodeling apparently does not take place, continue to show the presence of osteoporosis.

Convincing radiographic evidence of reversibility has not been shown for osteoporosis in adults. Although balance studies indicate that estrogen may arrest osteoporosis in postmenopausal individuals (3), an improvement in the radiologic appearance of the vertebrae has not been observed by members of Dr. Albright's group despite long-term use of estrogen in many cases. There is only a single case in the literature (4) in which radiographs suggest that three years of estrogen treatment may have led to improvement.

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THE EFFECT OF FOOD ON THE 3-HOUR THYROIDAL UPTAKE OF I^{131} IN HUMAN SUBJECTS*

TO THE EDITOR:

The thyrotropin (TSH) stimulation test provides a useful measure of thyroid function, especially in certain obscure cases of hypothyroidism (1, 2). In this test the proportions of a tracer dose of radioactive iodine that

* This investigation was supported by a grant from the Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service (PHS Grant A 565).

are taken up by the thyroid gland in three hours and the levels of serum protein-bound iodine (PBI) are measured both before and after administration of TSH. Because of previous observations that the presence of food in the stomach delays the absorption of iodide (3–5), patients are fasting when the I^{131} tracers are given. If this precaution were unnecessary the test would be more convenient, and hence a study was undertaken to reassess the effect of ingestion of food upon the 3-hour thyroidal I^{131} uptake in human subjects.

METHOD

Convalescent hospitalized patients were selected so as to include equal numbers of men and women. Their ages varied widely. The patients were divided into 2 groups: 1) a control group of 22 subjects, and 2) a study of group of 18 subjects. Both 3-hour and 24-hour thyroidal I^{131} uptakes were determined on two consecutive days. The control group fasted from 2 A.M. until the measurement of 3-hour uptake was completed on both days. Tracer doses of I^{131} were given at breakfast time—8 μc. the first day, and 24 μc. the second day. *In vivo* counting was performed with a scintillation counter, 15 cm. above the isotope standard, thyroid gland, and thigh. A count of the residual of the first tracer in the thyroid gland was made before the administration of the second tracer, and suitable corrections for decay of the first tracer were made at the time of the 3-hour and 24-hour count of the uptake of the second tracer. Patients in the study group were given the ordinary hospital breakfast on one of the two days (half were fed on the first day, and half on the second day); then food was withheld, as described for the control group on the other day. Thus, either an average change in uptake or a greater variation between uptakes on successive days due to presence of food could be detected.

RESULTS

The mean 3-hour thyroidal I^{131} uptake of the 22 control patients was 15.2 per cent on day 1 and 15.5 per cent on day 2; the mean difference between the two days was 0.3 per cent, and the variance of differences, 17.49. In the 18 patients of the study group, the mean 3-hour uptake of I^{131} on the day breakfast was given was 9.6 per cent, and on the day without food it was 10.1 per cent—a mean difference of 0.6 per cent. The variance of differences of this group was 17.88.

If the effect of food were to make the uptake more variable, the day-to-day differences and thus the variance of differences would be larger in the group fed on one of the two days than in the group receiving no food on both days. The fact that the variance of the differences in the study group (17.88) was so close to the variance of those in the control group (17.49)[1] indicates that following ingestion of food there was no greater variation in the 3-hour I^{131} uptake from day to day than existed in the fasting state.

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[1] $F = \frac{17.88}{17.48} = 1.0223$ (Ref. 6).

DISCUSSION

If, as has been reported, food in the stomach causes a delay in the absorption of iodine the 3-hour thyroidal I^{131} uptake should be depressed, and the 3-hour uptake differences in the study group should be larger than in the fasting group. In this investigation, however, there was no significant difference. Several reports help to explain this apparent paradox. Quetsch (3) in 1883 reported that oral iodide could be identified in the urine nine to eighteen minutes after ingestion by a fasting subject; a meal caused "moderate delay." More recently, Perkin and Lahey (7), in 108 fasting patients given 47 mg. of iodine as Lugol's solution, found that a peak plasma concentration was reached by half an hour to one and a half hours. Hamilton (8) stated that iodide appeared in the blood in three to six hours in the fasting patient. Hamilton and Soley (9) used fasting subjects who were given breakfast one and a half hours after I^{131}; there was no effect on the smooth uptake curves. Skanse (3) noted, in 3 fasting patients who vomited at one to one and a half hours after ingestion of radioiodine, that no radioactivity was found in the emeses. It would thus appear that in fasting subjects, absorption is usually complete by one and a half to two hours.

Myant and co-workers (4) and Stanley and Astwood (5) reported a 30-minute delay in the accumulation rate or gradient when iodide was administered to subjects shortly after breakfast. After the 30-minute delay, however, the gradient could be calculated, and food apparently did not affect the 3-hour or 24-hour uptakes.

It is not surprising then, that food had no effect on the thyroidal I^{131} uptakes in the present study. For if iodine absorption is complete in one and a half to two hours in fasting subjects, and food delays absorption only thirty minutes, practically all of the tracer I^{131} will be absorbed within two to two and a half hours in most non-fasting subjects. Thus a relatively slight delay in the gastro-intestinal absorption of I^{131} would not be expected to alter the 3-hour thyroidal I^{131} uptake significantly.

The subjects were fed typical hospital breakfasts: fruit juice, cereal or an egg (or both), toast, and tea or coffee. The portions were not large and the meals would be rated as "light." Heavy meals may have a greater delaying effect; data from Storaasli (unpublished) support this view. It seems unnecessary, therefore, to withhold food from patients for the TSH test, since the data of the present study show that a light meal has no significant effect. It is probably best to withhold food if accumulation rate or clearance studies are to be performed.

SUMMARY

The 3-hour thyroidal I^{131} uptake was studied in fasting and in lightly fed subjects. No significant change in uptake was demonstrated. It is concluded that patients may eat a light breakfast before thyroidal I^{131} uptake studies involving periods of three hours or longer.

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February 17, 1958

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The Endocrine Society

TENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE STATE UNIVERSITY OF NEW YORK, UPSTATE MEDICAL SCHOOL

SYRACUSE, N. Y.

September 29 through October 3, 1958

For information concerning the program and registration, write to *Charles W. Lloyd, M. D., State University of New York, Upstate Medical Center, Syracuse 10, N. Y.*

The registration fee is $100.00. Residents and Fellows will be admitted for a reduced fee of $30.00. Those wishing to reserve rooms should communicate directly with the Hotel Syracuse.

(For program, see the July issue of the Journal)

THE 1959 ANNUAL MEETING

The Forty-First Annual Meeting of The Endocrine Society will be held in the Haddon Hall Hotel, Atlantic City, New Jersey, Thursday, Friday, and Saturday, *June 4, 5, 6, 1959.*

The Chairman of Local Arrangements is Dr. Matthew Molitch.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 5, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Chalfonte-Haddon Hall Hotel will hold 300 bedrooms for members until May 1, 1959, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations now directly with the hotel, advising them of time and date of arrival and departure. Avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 1, 1959 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Frank Engel, Duke Hospital, Durham, North Carolina, not later than *February 1, 1959. It is imperative that the abstracts be informative and complete with results and conclusions*—not a statement that those will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately two hundred abstracts submitted each year and the compiling of the program from these involves a tremendous amount of time and effort on the part of the Program Committee. The Council requests that the authors adhere strictly to the following rules when submitting abstracts, otherwise they cannot be considered:

1. *It is assumed that abstracts submitted for this program have not and will not be submitted elsewhere.*
2. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of the nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
5. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

During the past year a substantial legacy has been bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is to be the highest honor of The Endocrine Society and is to be represented by a medal that is to be known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award will replace the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J. B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional

promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15, 1958.*

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15, 1958.*

The American Goiter Association

RECIPIENTS OF THE 1958 AWARDS

THE VAN METER PRIZE AWARD

Dr. Duncan D. Adams of Dunedin, New Zealand, has been named the winner of the Van Meter Prize Award for 1958. The title of his paper was "The Presence of an Abnormal Thyroid-Stimulating Hormone in the Serum of Some Thyrotoxic Patients (*J. Clin. Endocrinol. & Metab. 18:* 699 (July) 1958).

Dr. Adams was born in New Zealand in 1925. He received his medical education at the University of Otago Medical School, and graduated with the degree of M.B., Ch.B. in 1950. From 1951 to 1953 he held a Research Fellowship of the New Zealand Medical Research Council, working under the direction of Dr. H. D. Purves. In 1954 he was lecturer in Prof. J. B. Collip's Department of Medical Research at the University of Western Ontario in Canada. Since 1955 he has been a Research Officer in the New Zealand Medical Research Council's Endocrinology Research Department.

AWARD FOR LONG AND MERITORIOUS SERVICE

The Committee appointed to selected a member of the Association to receive its Award for Long and Meritorious Service unanimously chose *Dr. Richard B. Cattell* of the Lahey Clinic, Boston, Massachusetts.

Dr. Cattell was born in Martins Ferry, Ohio, in 1900. He received the degree of A.B. from Mt. Union College, Alliance, Ohio, in 1921 and the M.D. degree from Harvard Medical School in 1925. He served his internship in St. Luke's Hospital, New York City, 1925–1927; was a Fellow in Surgery, Lahey Clinic, Boston, 1927–1929; on the Staff of the Lahey Clinic from 1927 on; and Director of the Lahey Clinic from 1953 to the present. He served as a private in the Medical Corps, USA, in World War I (1917–1919), and as Honorary Consultant, Bureau of Medicine and Surgery, USN, 1942–1952; 1956—. He is Surgeon-in-Chief, New England Baptist Hospital; Chairman, Medical Administrative Board, and Surgeon, New England Deaconess Hospital; and Surgeon, Robert Breck Brigham

Hospital, Parker Hill Medical Center, Booth Memorial Hospital, and Brooks Hospital. He became a Diplomate of the American Board of Surgery in 1937, is a Fellow of the American College of Surgeons (Member Board of Governors 1955–1957; Regents, 1957—), a member of many other surgical and medical societies, and was President of The American Goiter Association, 1954–1955.

5TH RÉUNION DES ENDOCRINOLOGISTES DE LANGUE FRANÇAISE

The 5th Reunion of Endocrinologists of the French Language will take place in *Paris, France,* on *June 29–30 and July 1, 1959.* The subjects will be: I. Exploration of Thyroid Function (J. Decourt, G. Dreyfus, J. Roche, J. Mahaux, J. Lederer, A. Vanotti, C. P. Leblond and C. Cassano). II. The Adrenal Medulla: Physiology; Pheochromocytoma (C. DuBost, H. Hermann, J. Malmejac, D. Picard and A. Soulairac). Further information may be obtained from Dr. A. Netter, Secretary-General of the Société d'Endocrinologie, Hôpital Necker, 149 rue de Sèvres, Paris 15, France.

FOURTH INTERNATIONAL CONGRESS OF CLINICAL PATHOLOGY (CLINICAL ANALYSIS)

The Fourth International Congress of Clinical Pathology will be held in *Madrid, Spain, June 13–17, 1960.* All physicians interested in clinical analysis in its various aspects (biology, pathology) may take part in this Congress. Further information may be obtained from the Secretary of the Organization Committee, Dr. José Aparicio Garrido, Facultad de Medicina, Pabellón Nr. 2, Ciudad Universitaria, Madrid, Spain.

GRANTS-IN-AID

The Ortho and the Carl G. Hartman grants-in-aid of the American Society for the Study of Sterility, each of $500.00, are available for 1959. Applications for these 2 grants-in-aid should be sent to the Secretary of the Awards Committee of the American Society for the Study of Sterility, Dr. Robert B. Wilson, 200 First Street Southwest, Rochester, Minnesota, by *December 31, 1958.* No application will be considered by the committee unless it is accompanied by 5 copies of a brief outline of the research project for which the grant-in-aid of $500.00 is being sought.

BOOK REGISTER

Annales Instituti Obstetricii et Gynecologici Universitatis Helsinkiensis. Edited by AARNO TURENEN--PAAVO VARA. Volumes XXX and XXXI. These volumes contain numerous articles (many in English) covering research work by surgeons of the Women's Clinic during the years 1953 and 1954; 132 pages and 110 pages respectively; illustrated. University of Helsinki, Helsinki, Finland.

Atti del simposio farmaci antidiabetici. Contains papers (in Italian) presented at a sym posium on the most recent advances in antidiabetic therapy, held at the University of Siena, May 16–27, 1957. *Minerva Medica* (Turin, Italy), Vol. 49, pages 1391–1547, 1958.

(*The*) *Cerebrospinal Fluid: Production, Circulation and Absorption.* Ciba Foundation Symposium, edited by G. E. W. WOLSTENSHOME, M.A., B.Ch. and CECILIA M. O'CONNOR, B.Sc. Contributors are anatomists, neurologists, pathologists, psychiatrists, pharmacologists and neurosurgeons from all over the world, who attended this Symposium held in May 1957. Papers and discussions cover questions such as the structure and relationships of the arachnoid granulations, the fine structure of the mammalian choroid plexus, the nerves of the meninges of the choroid plexus, the production and circulation of the cerebrospinal fluid, the formation and absorption of the cerebrospinal fluid using radioactive isotopes, vitamins and the cerebrospinal fluid, some aspects of the relationship between the cerebrospinal fluid and the central nervous system, neuropathologic observations on the cerebrospinal fluid pathway, and clinicopathologic aspects of the cerebrospinal fluid circulation; 347 pages; 141 illustrations; tables; 1958. Little, Brown & Company, Boston, Massachusetts. Price $9.00.

Clinical Obstetrics and Gynecology. Vol. I. This first volume of a new quarterly book series contains the papers of a Symposium on *Fibromyomas of the Uterus,* edited by ROBERT A. KIMBROUGH, M.D. and a Symposium on *Toxemias of Pregnancy,* edited by LOUIS M. HELLMAN, M.D., constituting the March and June issues for 1958; 544 pages; illustrated. Paul B. Hoeber, Inc., 49 East 33rd Street, New York 16, N. Y. *Clinical Obstetrics and Gynecology* is available by subscription at $18.00 for four consecutive numbers.

Harofé Haivri (*The Hebrew Medical Journal*): *Thirtieth Anniversary Year.* Edited by MOSES EINHORN, M.D. Volumes 1 and 2 for 1957 include articles on such topics as The Health of Israel, Historical Medicine, Medicine and Religion, and Hebrew Medical Terminology. Editorial Office—983 Park Avenue, New York, N. Y.

Hormone Production in Endocrine Tumours. Vol. XII of Ciba Foundation Colloquia on Endocrinology, edited by G. E. W. WOLSTENHOLME, M.A., B.Ch. and MAEVE O'CONNOR, B.A. Included is a cumulative *Index* to all 12 of the colloquia in this series. The volume contains a summary of the important work on hormone-producing tumors during the last twenty-five years. The new biochemical micromethods have produced many new biochemical facts concerning these tumors. These are correlated with previous data. A partial table of contents includes: Experimental Pituitary Tumours, Induction of Pituitary Tumours and Mela-

nomas in the Golden Hamster, Normal and Abnormal Iodinated Compounds in the Serum of Subjects with Carcinoma of the Thyroid, Goitrogen-Induced Thyroid Tumours, Biosynthesis of Steroids in Hyperactive and Tumour-Bearing Human Glands, Production of Oestrogenic Hormones by Granulosa Cell Tumours in Mice, Hyperplasia and Tumours of the Human Adrenal Cortex (Histology, Enzymic Changes and Corticoid Production), Tumours of the Adrenal Cortex, Consideration of Some Types of Adrenal Tumours, and Some Studies of Ovarian Tumorigenesis; 364 pages; 58 illustrations; 1958. Little, Brown & Company, Boston, Massachusetts. Price $9.00.

(*Les*) *Hypercorticismes surrénaliens: signes cliniques et biochimiques, physiopathologie, indications thérapeutiques.* By É.-É. BAULIEU, Chef de Clinique Médicale et chef des travaux de Chimie à la Faculté de Medecine de Paris. Part I is concerned with the general questions of the physiology and biosynthesis of the adrenocortical hormones, the role of ACTH, catabolism, and principles and techniques regarding the adrenal steroid hormones. Part II deals with hypercorticism—Cushing's syndrome, adrenogenital syndrome, hyperaldosteronism and other forms of the disease. The book outlines methods for precise diagnosis, which is essential to the establishment of successful therapeutic procedures; 142 pages; 25 figures, 1958. Masson et Cie, Éditeurs, Paris, France. 1.400 fr.

L'Année endocrinologique. By M. ALBEAUX-FERNET, L. BELLOT, P. BUGARD, J. CHABOT, J. DERIBREUX, M. GELINET, and J. D. ROMANI. This tenth volume in the series covers the latest advances in endocrine-metabolic disorders, endocrine function, the neuro-hypophysis, the ovaries, the parathyroids, the mammary gland, senescence, polyendocrine syndromes, the adrenals, the testes, the thyroid, and general endocrine therapy; 240 pages; 1958. Masson et Cie, Éditeurs, Paris, France.

Le Tireopatie. Vol. VI. Il Gozzo. (In Italian) Transactions of the Third Congress on Diseases of the Thyroid, Torino, Italy, July 1957. This book deals with various clinical and experimental aspects of goiter and cretinism, including studies in Italy, Argentina, and Slovakia. There are several articles on endemic goiter; 325 pages; illustrations; tables; 1958. Minerva Medica, Torino, Italy.

Psychiatric Research Reports of the American Psychiatric Association, 1700 18th Street, N.W., Washington 9, D. C. Editors: NATHAN S. KLINE, M.D., ROBERT A. CLEGHORN, M.D., MILTON GREENBLATT, M.D., HAROLD E. HIMWICH, M.D., WILLIAM T. LHAMON, M.D. and BENJAMIN PASAMANICK, M.D.

Research in Affects. Psychiatric Research Report No. 8 of the American Psychiatric Association. APA Regional Research Conference, State University of New York, Upstate Medical Center, Syracuse, N. Y., April 5–6, 1957. Four symposia deal with one of the most important current topics in psychiatry, as follows: Disorders of Affects During Childhood; Communication and Affects; Experimental Studies on Affects; and Drugs and Affects; 186 pages; 1957. Price $2.00.

Research in Psychiatry with Special Reference to Drug Therapy. Psychiatric Research Report No. 9 of the American Psychiatric Association. APA Regional Research Conference, the Medical Schools and Affiliated Hospitals of Philadelphia, Pa., Nov. 16–17, 1956. This Conference was outstanding for its intensive evaluation of the new drug, proclorperazine, for a distinguished academic lecture (Some Problems in Experimental Psychiatry) and for vigorous and controversial discussion; 181 pages; 1958. Price $2.00.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Office, 16 West 46th Street, New York 36, N. Y.

Gestation. Fourth Conference, March 5–7, 1957, Princeton, N. J. Edited by CLAUDE A. VILLEE, Ph.D., Associate Professor of Biological Chemistry, Harvard Medical School, Boston. The subjects included in this volume are: The Physiology of Fetal Fluids, The Origin of Amniotic Fluid, The Morphologic Manifestations of Uptake of Materials by the Yolk Sac of the Pregnant Rabbit, and The Development of Fetal Immunity; 216 pages; 64 figures; 20 tables, 1958. Price $4.50.

Glaucoma. Second Conference, Dec. 3–5, 1956, Princeton, N. J. Edited by FRANK W. NEWELL, M.D., Dept. of Ophthalmology (Surgery), University, of Chicago, Chicago, Illinois. The subjects covered in this volume are: The Historical Background of Mechanisms Concerned with Aqueous Formation, An Introduction to Mathematical Formulation of Aqueous Dynamics, Mechanisms of Transport by Membranes, The Glaucoma Problem, and Applanation Tonometry, 245 pages; 63 figures; 29 tables; 1957. Price $4.95.

University of Colorado Studies, Series in Biology No. 4. This volume contains 5 papers dealing with several aspects of normal and pathologic growth processes that are considered significant in the investigation of the causes of cancer and arthritis. The 5 articles are entitled: Effect of Mast Cell Products on Capillary Permeability, Serotonin as a Mast Cell Product, Significance of Peroxidase in Eosinophils, The Thymus in Nucleohistone Metabolism, and Neuronal Trephocytes; 158 pages; 1958. University of Colorado Press, Boulder, Colorado. Price $3.00.